Energetics in signaling and development

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Molecular Biophysics & Biochemistry

KITP
Santa Barbara
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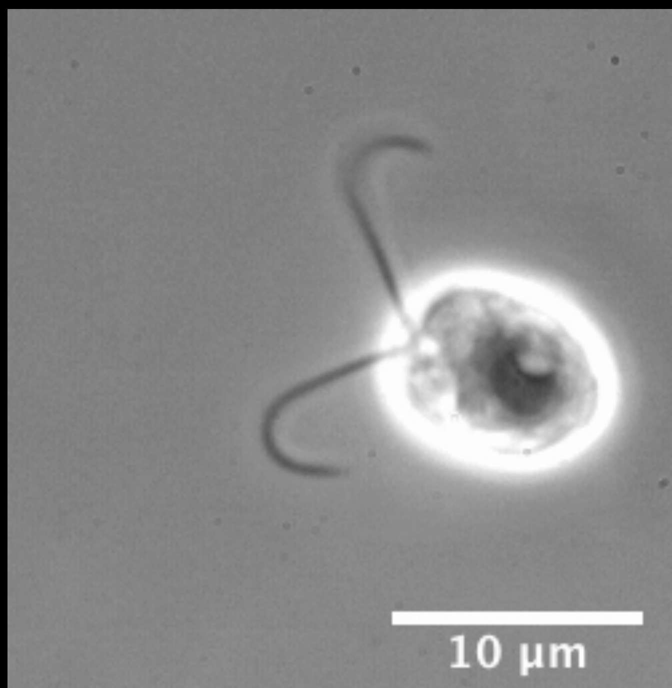
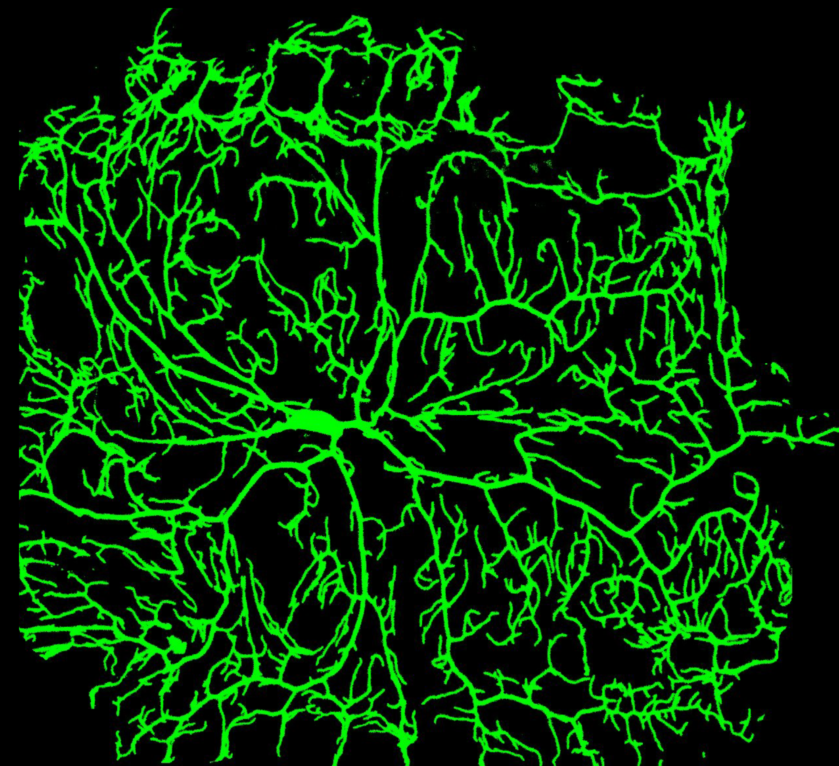
Dynamic morphology of cells

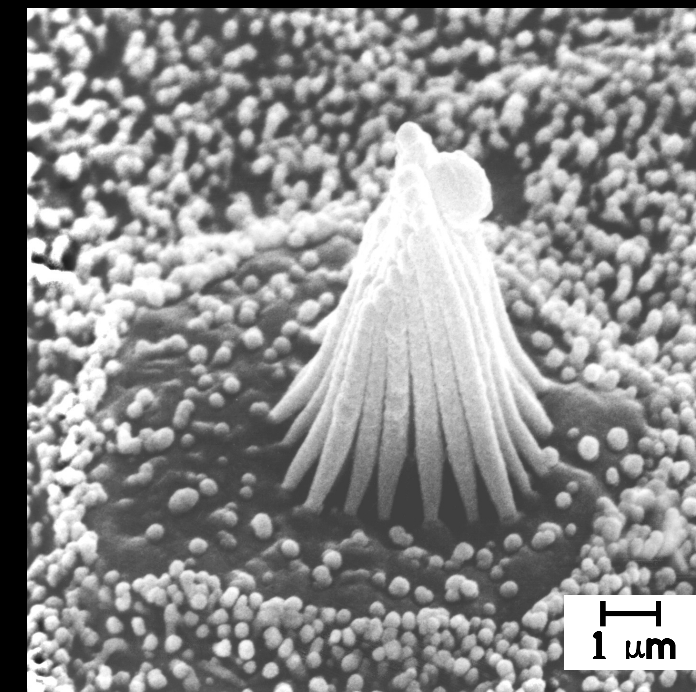
Cilia and flagella



Neuron



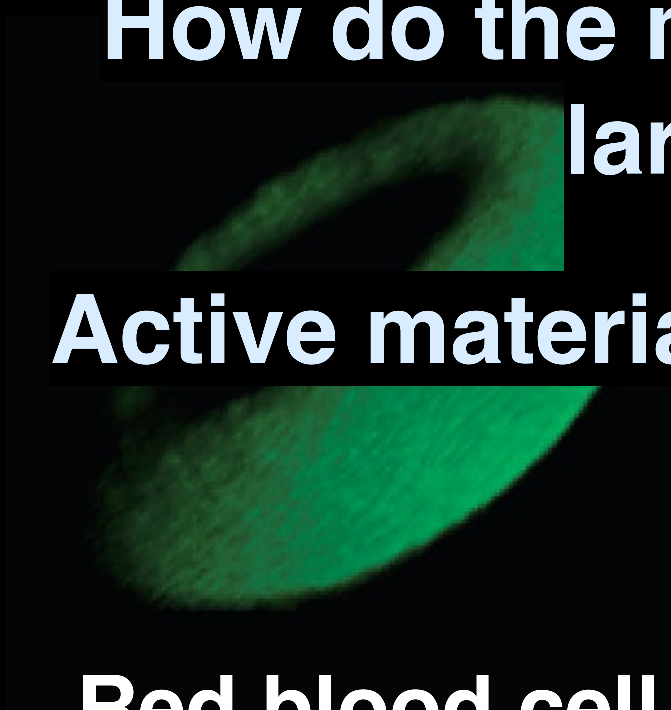
Hair bundle



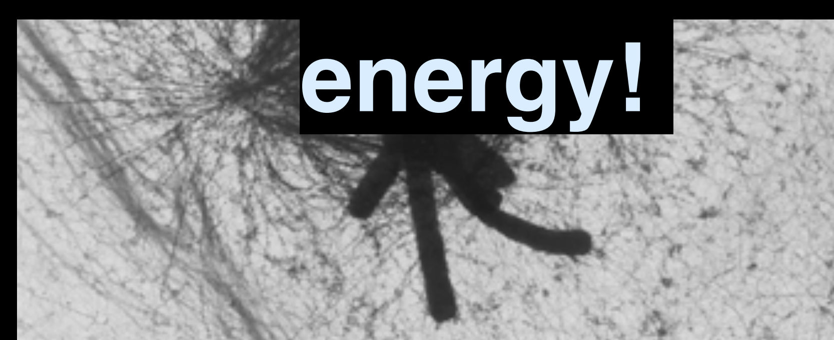
(8 µm high)

How do the molecules build and move structures much larger than molecular dimensions?

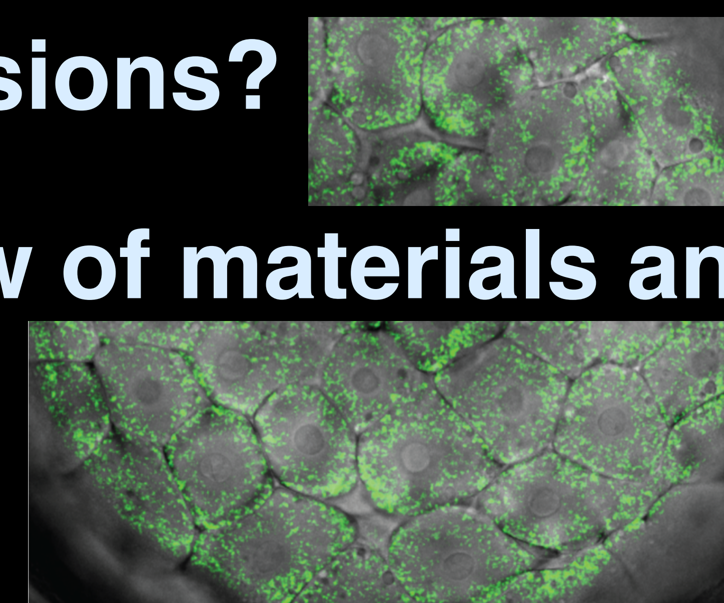
Active materials: there is a constant flow of materials and energy!



Red blood cell
(8 µm across)



Mitotic spindle
(10 µm pole-to-pole)



Zebrafish embryo
(800 µm diameter)

Two energetic stories

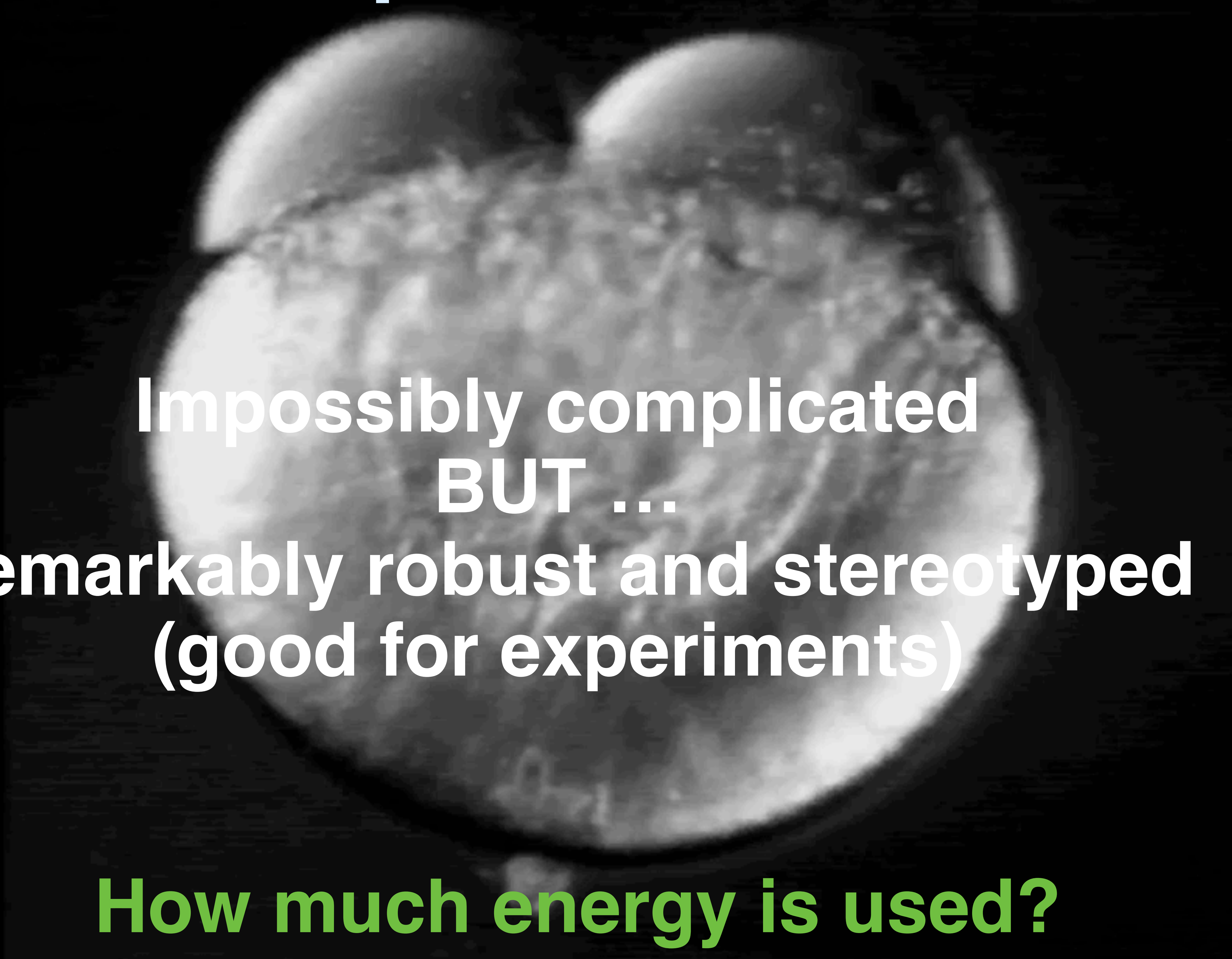
1. Early development in zebrafish

2. Molecular motors

1:fr10391 ← 8 s counter:

Time is from 0:45 to 17:00 hrs post fertilization (nonlinear replay speed)

Development of the fish



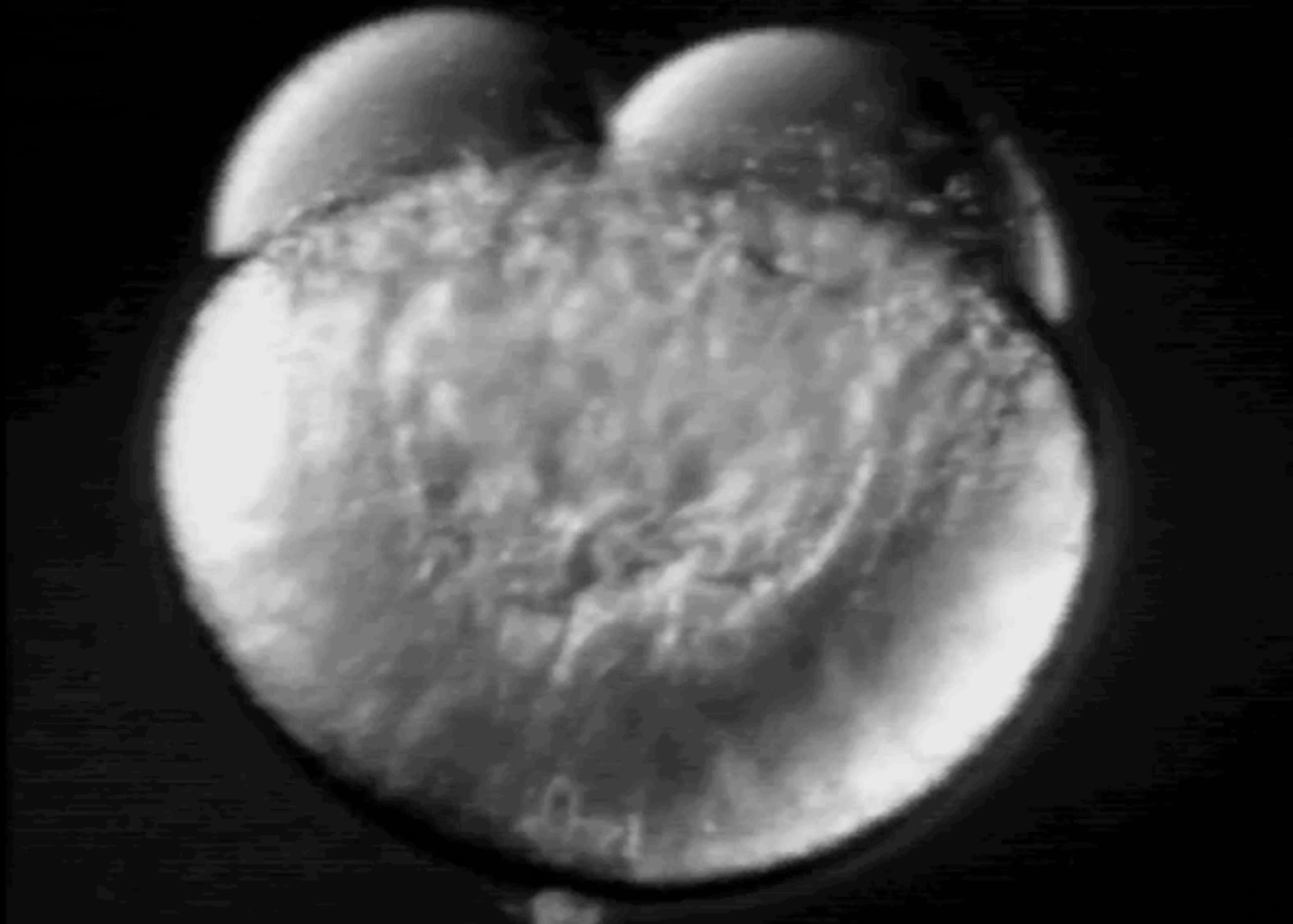
Impossibly complicated
BUT …
Remarkably robust and stereotyped
(good for experiments)

How much energy is used?

Movie: RO Karlstrom & DA Kane, Development 1996

Reductive cleavage stage

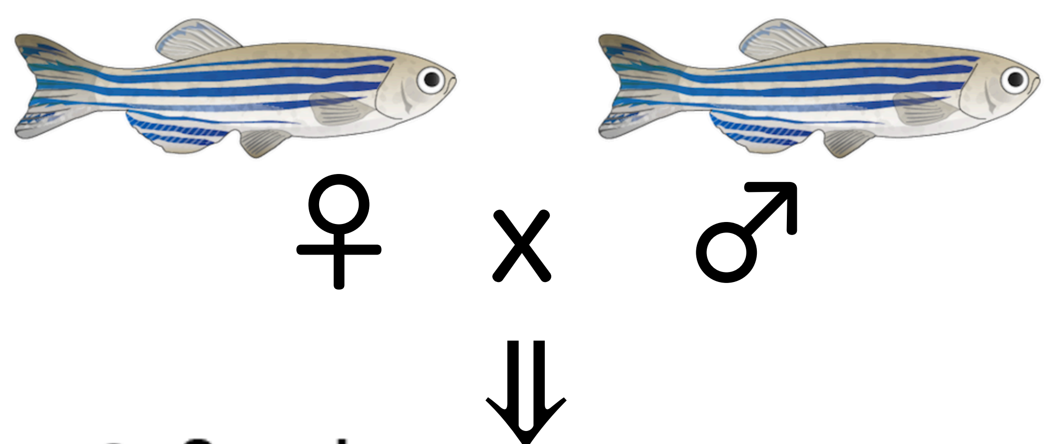
(Early divisions **without growth**)



Synchronous up to the tenth division

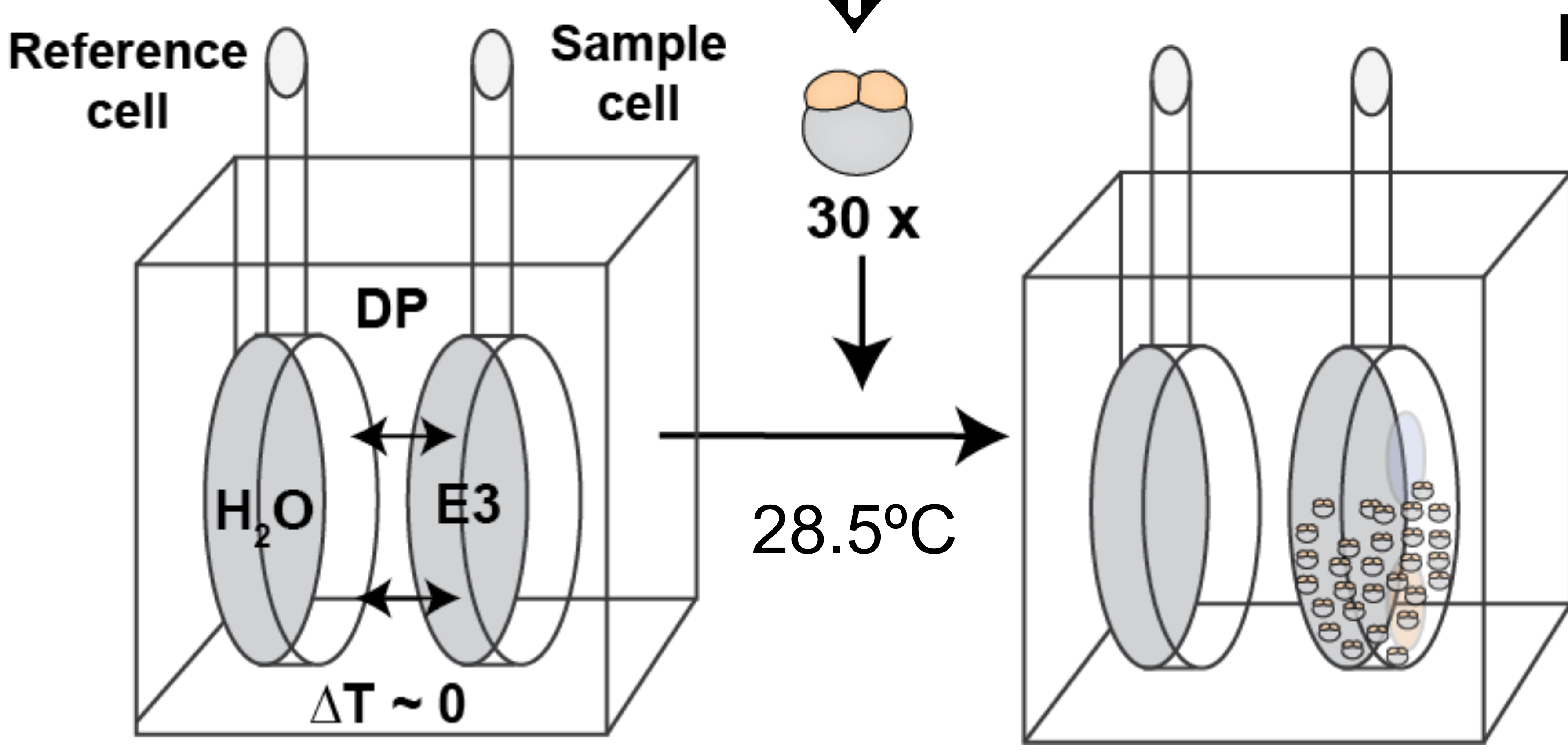
Movie: RO Karlstrom & DA Kane, Development 1996

Measurement of embryonic heat flow by isothermal calorimetry



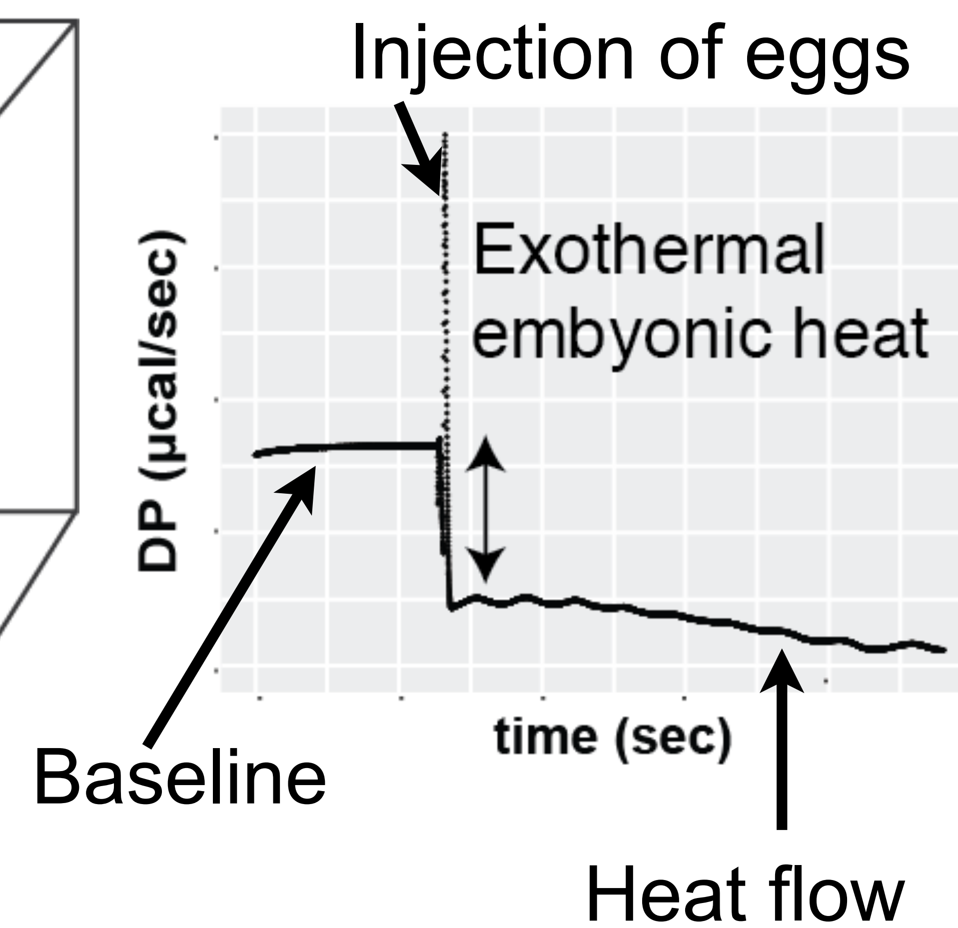
Jonathan Rodenfels

Karla Neugebauer

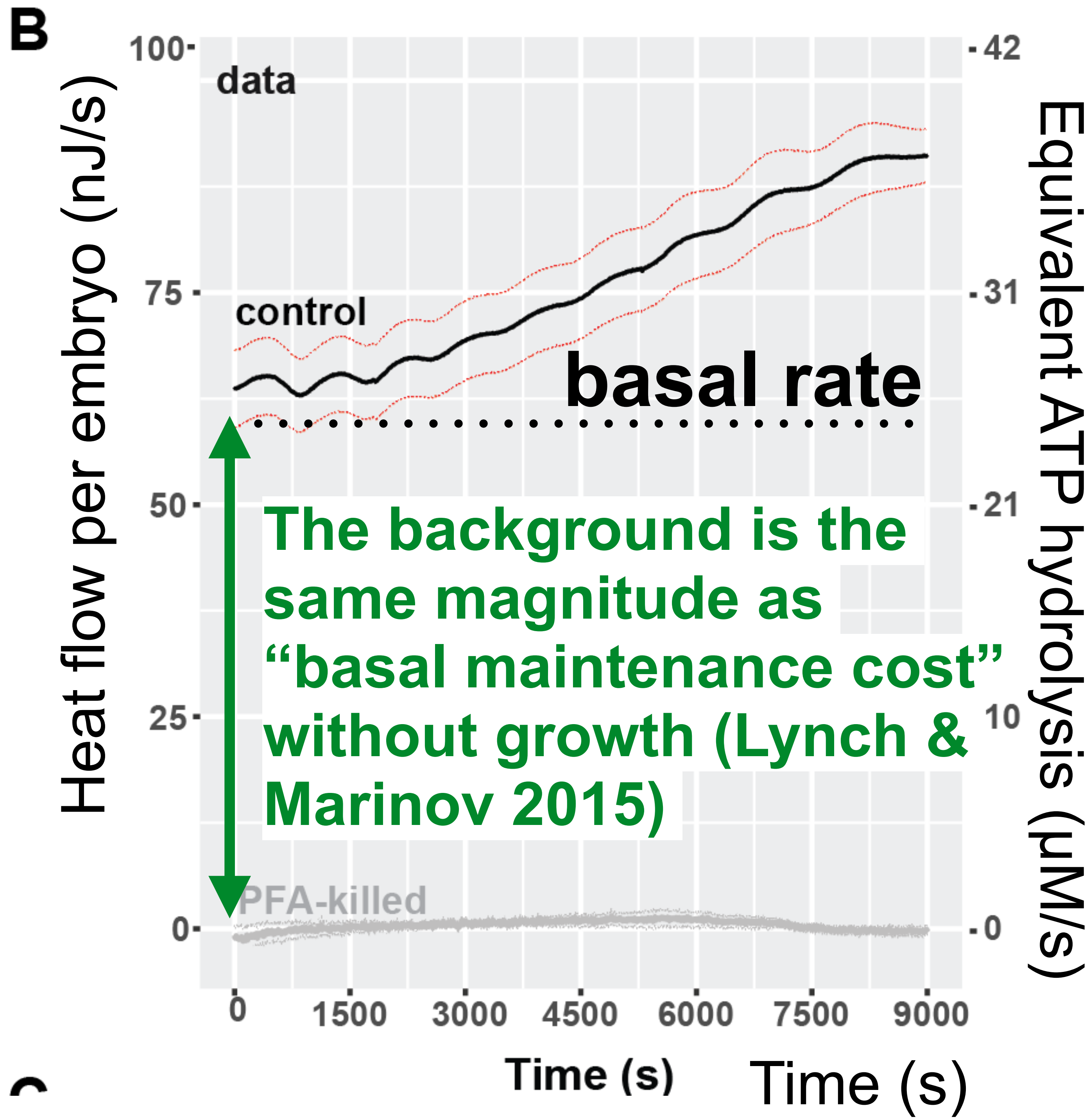


Malvern MicroCal VP-ITC (usually used for protein-protein interactions

Eggs staged at 2-cell cleavage (within 3 minutes)



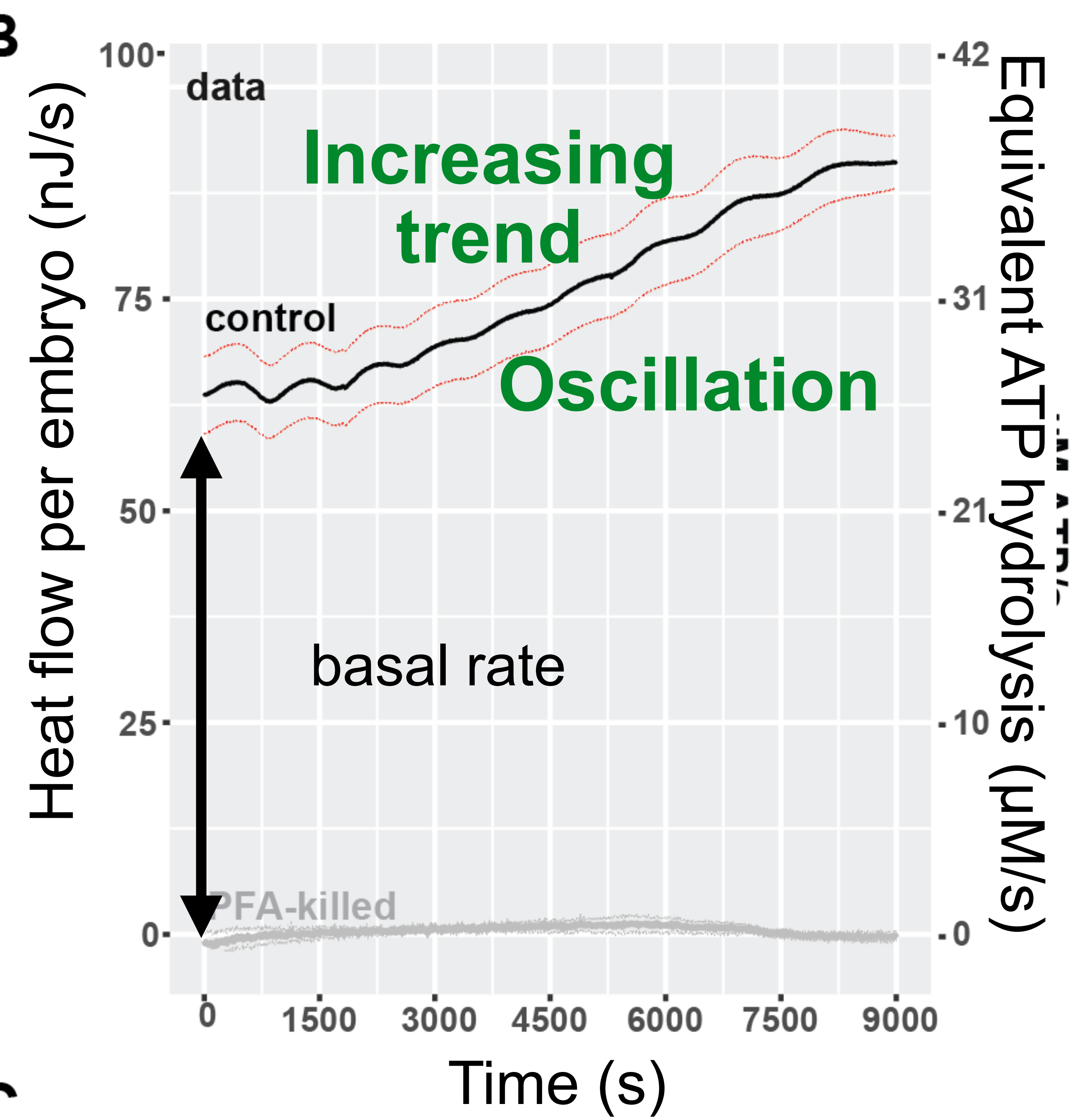
Embryogenesis is exothermic:
Heat flows out of the embryo

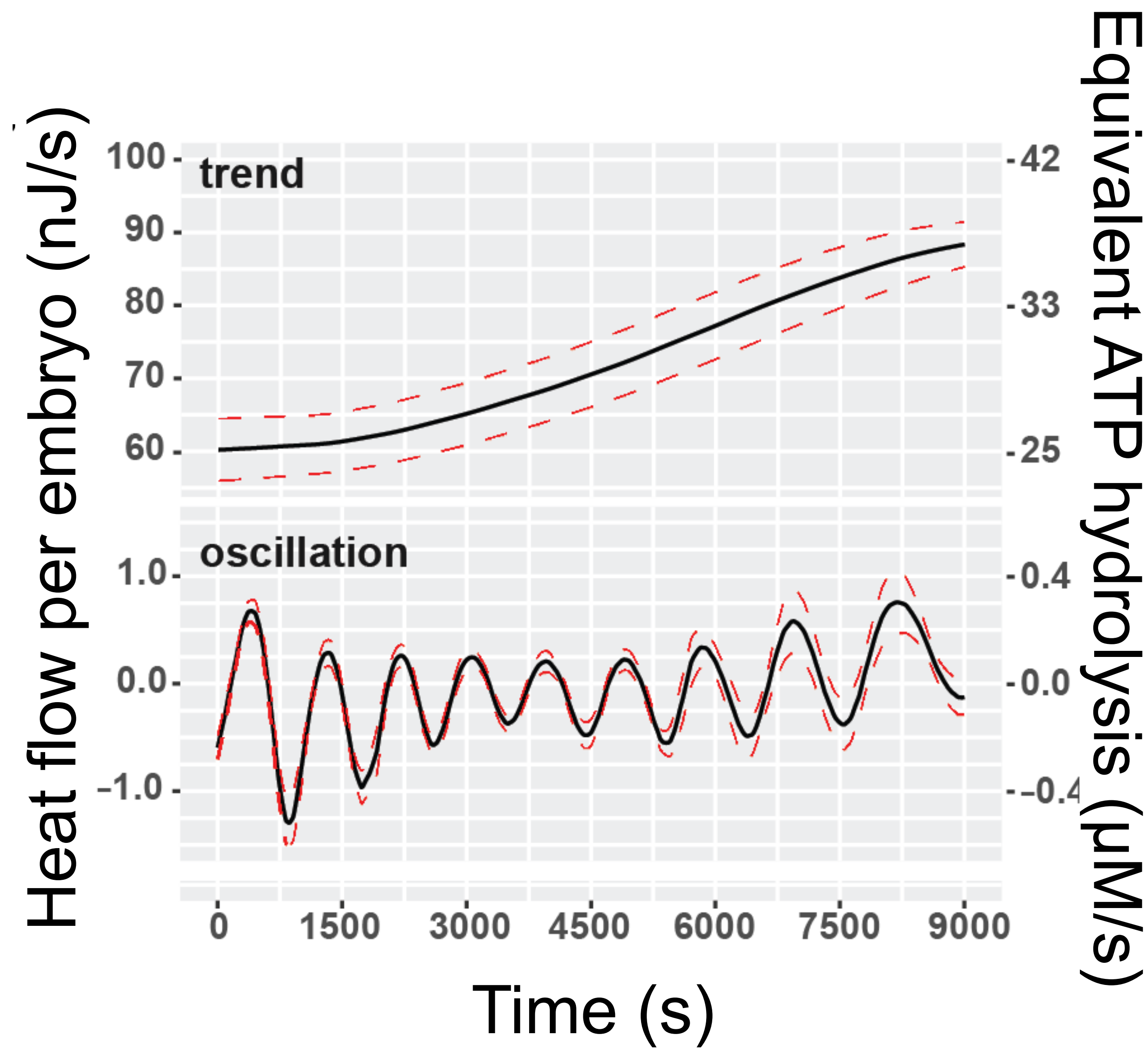


Similar to the "basal metabolic rate" defined by West and many others) of fish and humans at rest of 1 W/kg

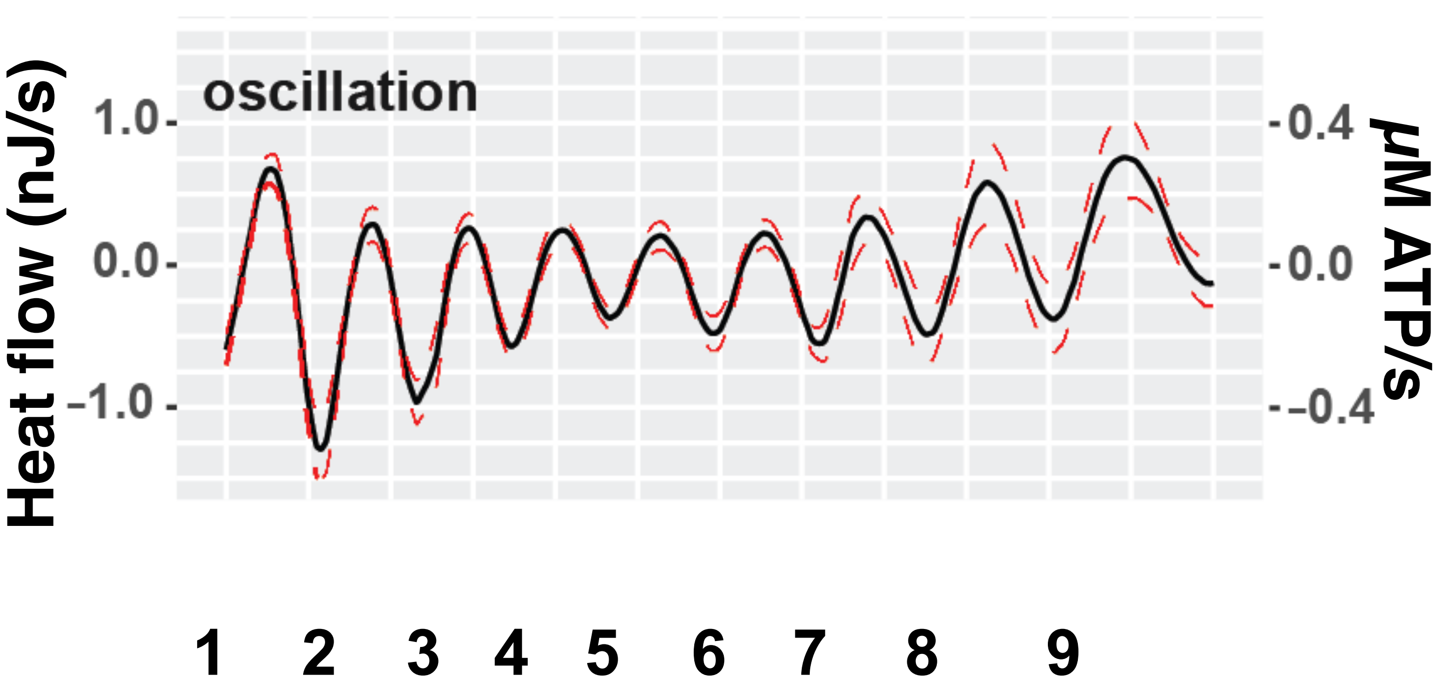
Heat flow measures the enthalpy changes of all biochemical reactions in the embryo

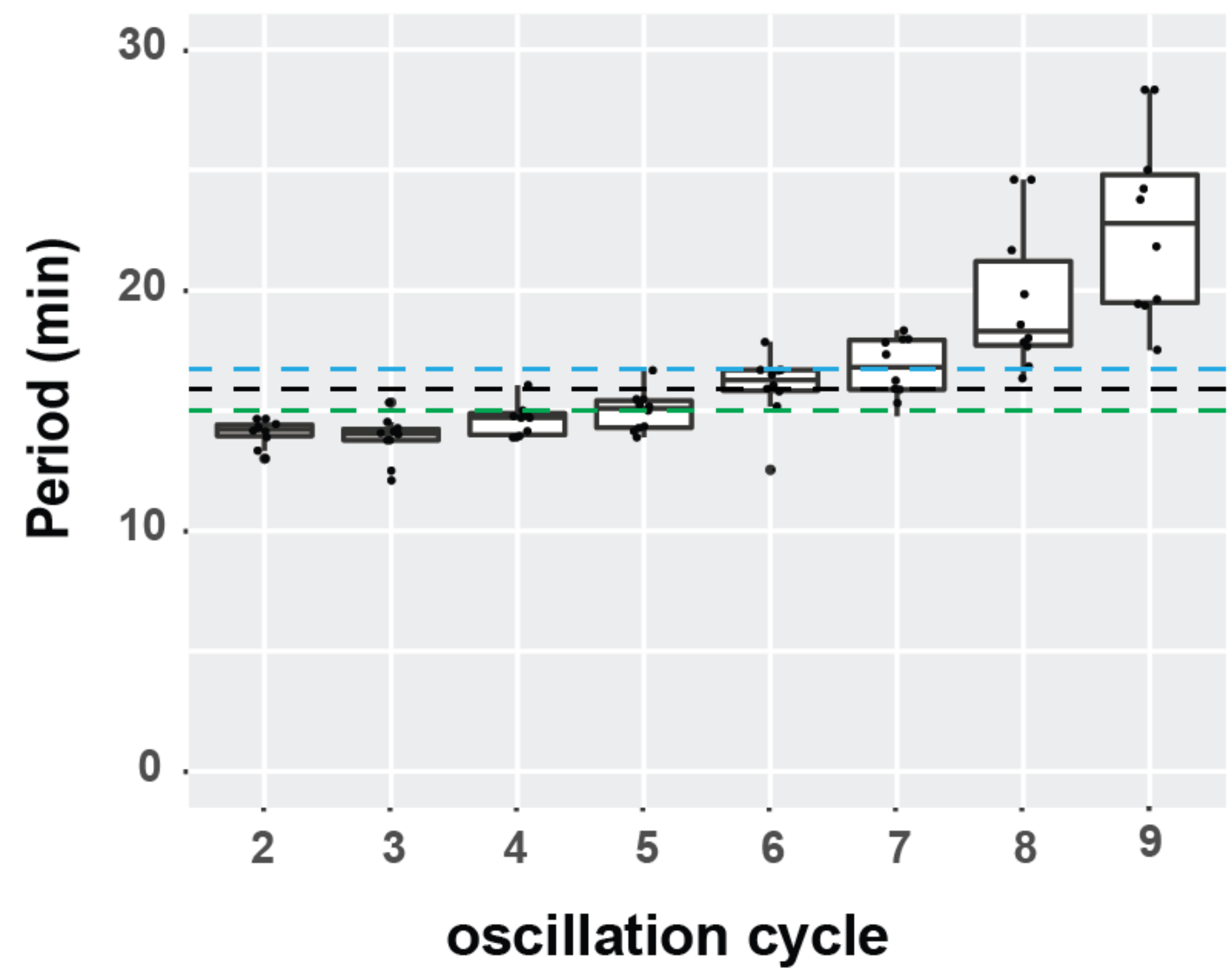
Two other components





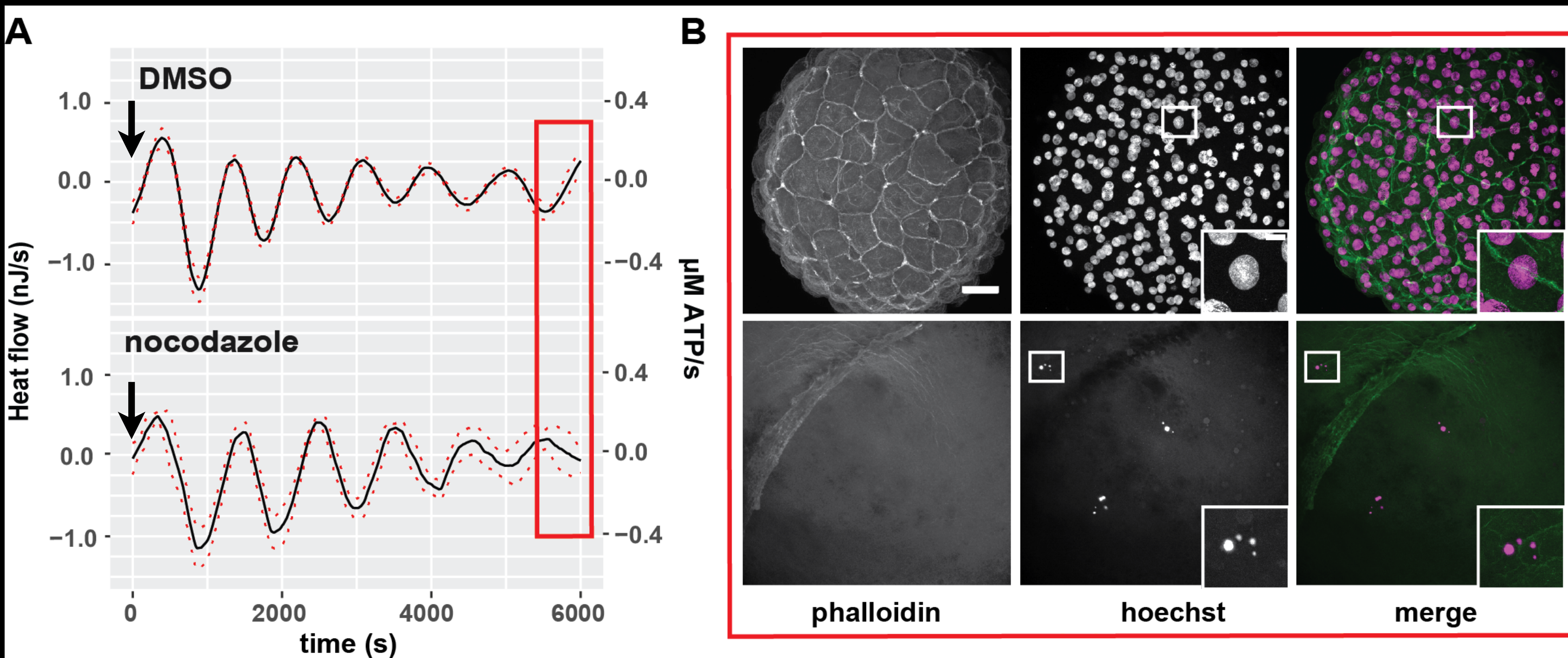
The oscillatory component has the same period as the cell cycle





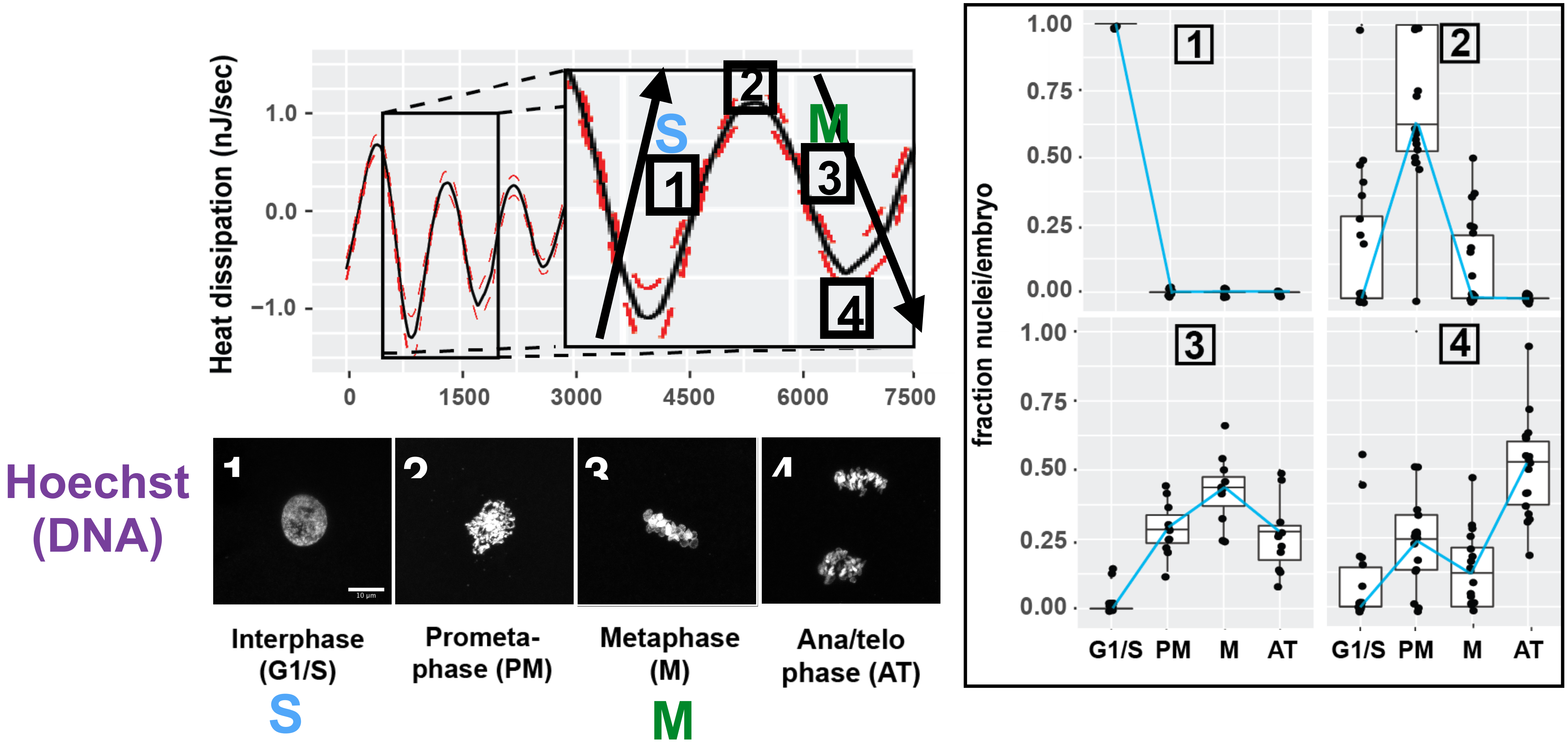
The cell cycle period is roughly 15 minutes, increasing with cycle number

The oscillatory component does not depend on DNA Replication, mitosis or cell division



Nocodazole blocks DNA replication and mitosis

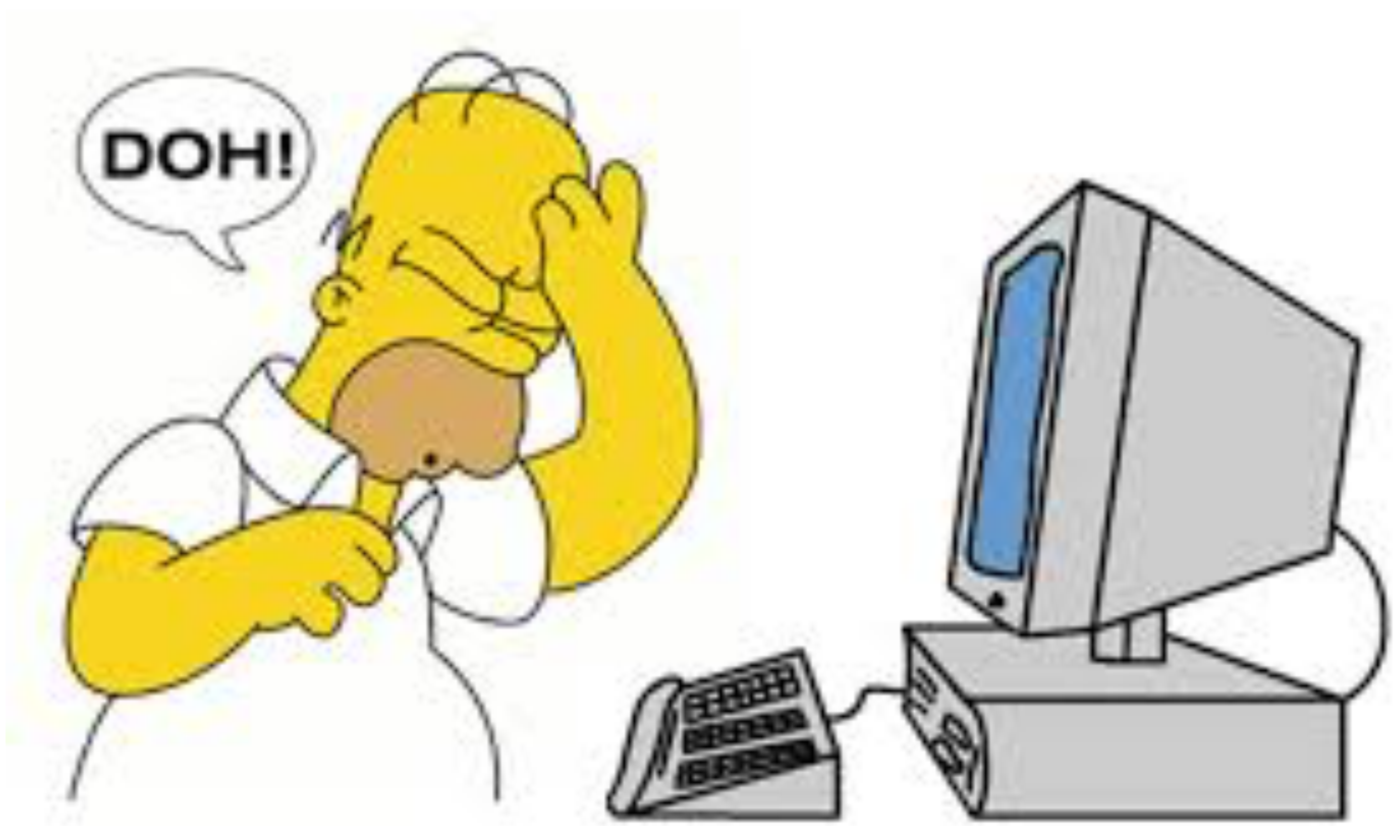
Embryonic heat flow peaks during mitotic entry and troughs during mitotic exit



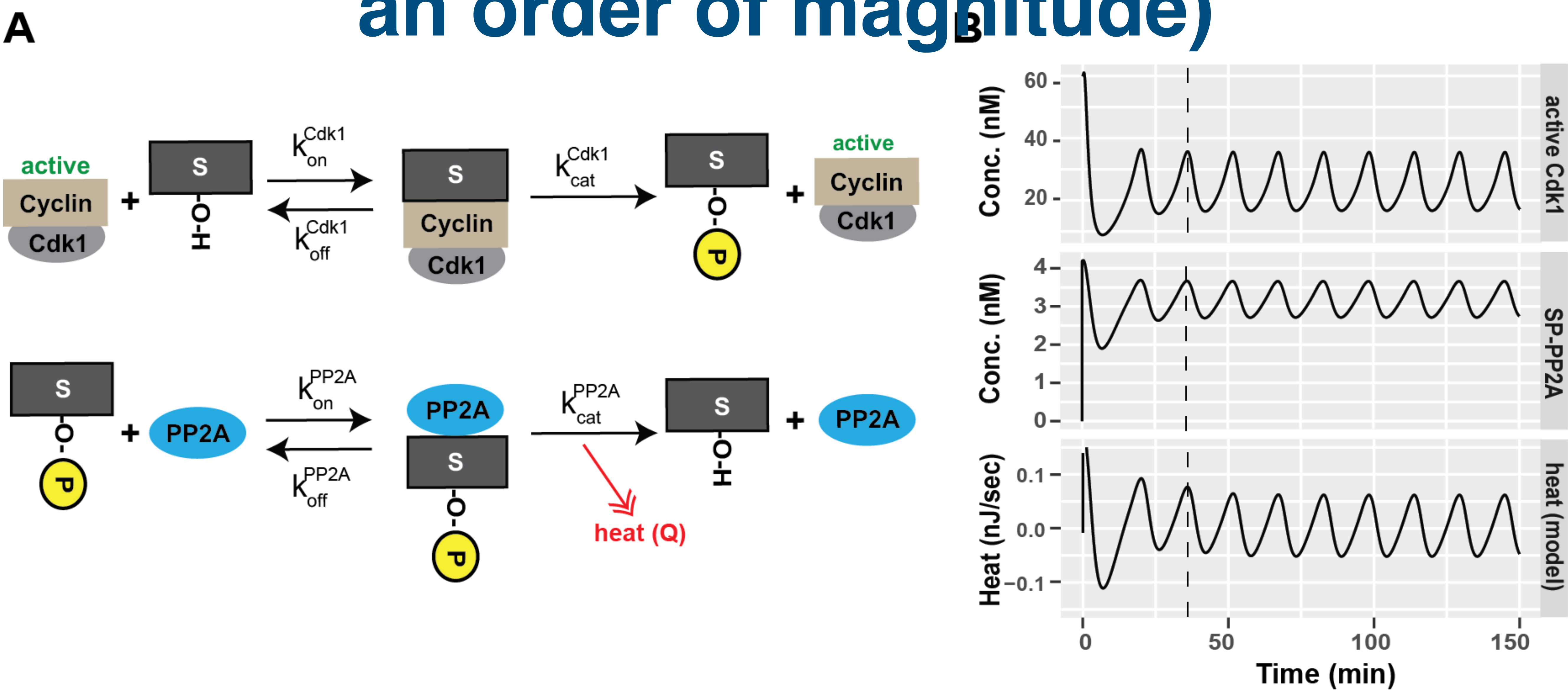
Not in phase with either replication or cell division!?

Heat cannot be produced significantly by DNA replication or mitosis!

If it were either, then the oscillatory component would increase exponentially in amplitude



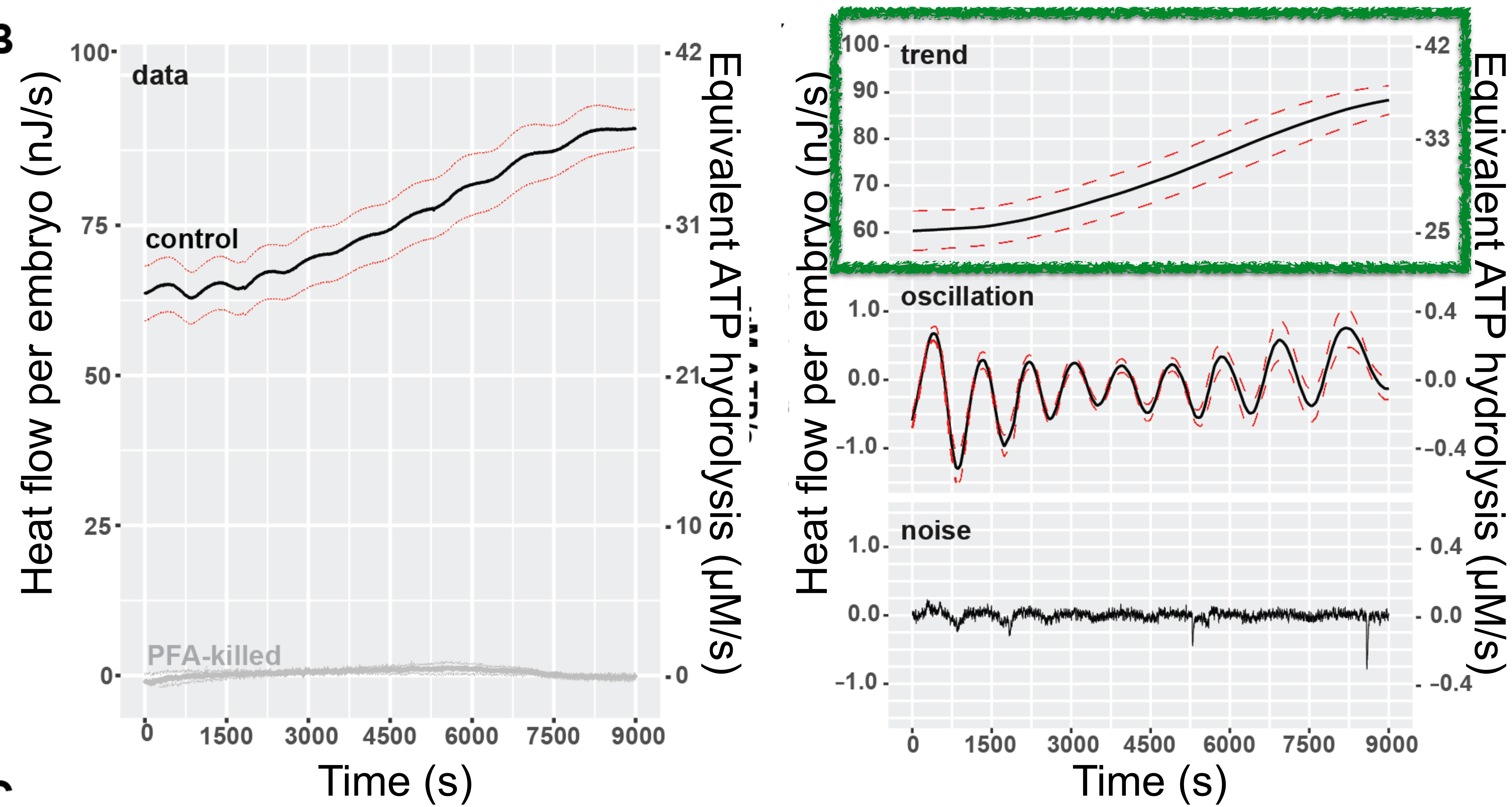
Modeling shows that protein phosphorylation and dephosphorylation has the right phase and amplitude (within an order of magnitude)



Model after Tsai, Theriot and Ferrell (2014). PLoS Biol 12, e1001788–15.

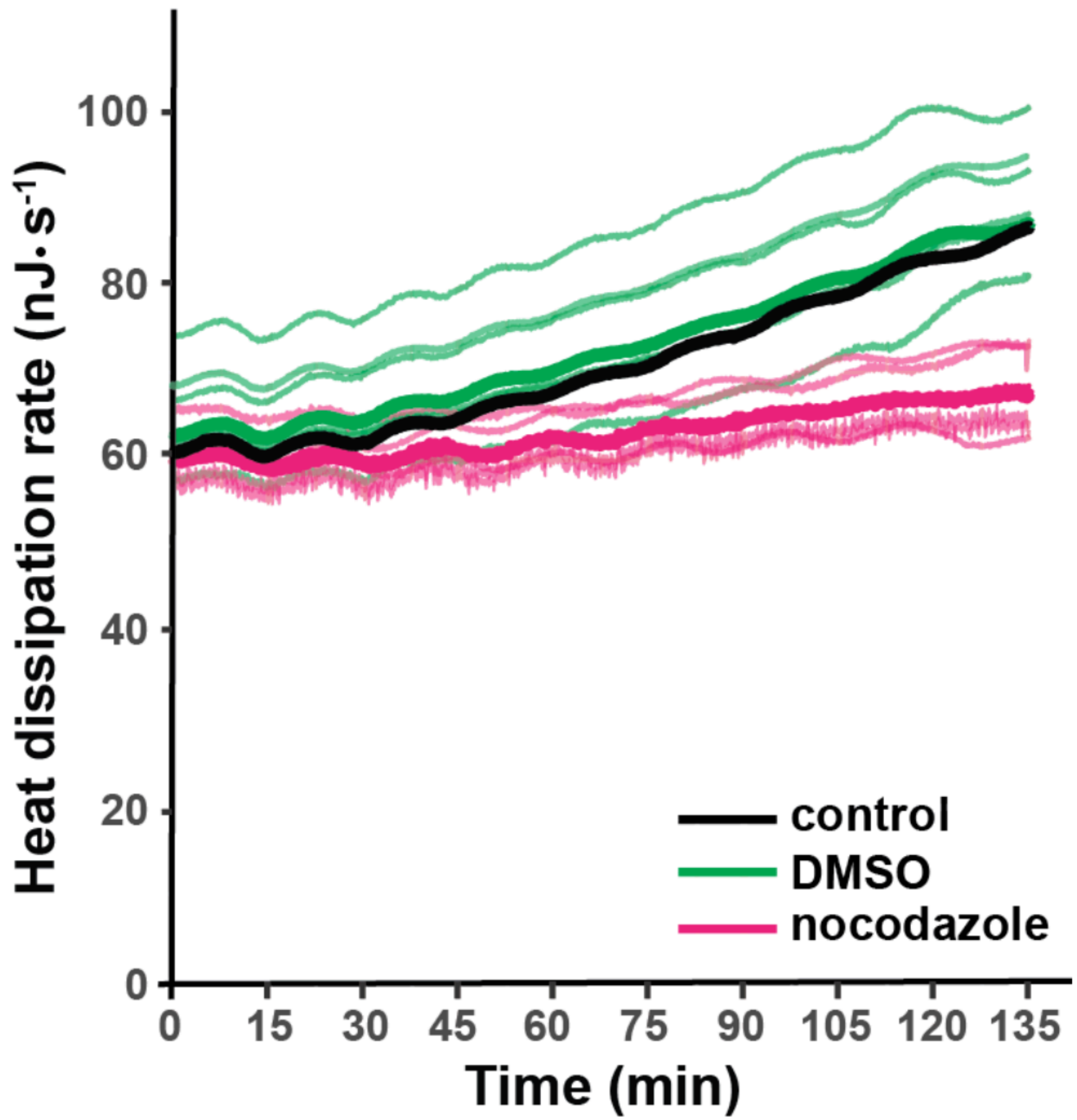
Question: How much energy do you need to run a clock accurately?

What about the slow component?



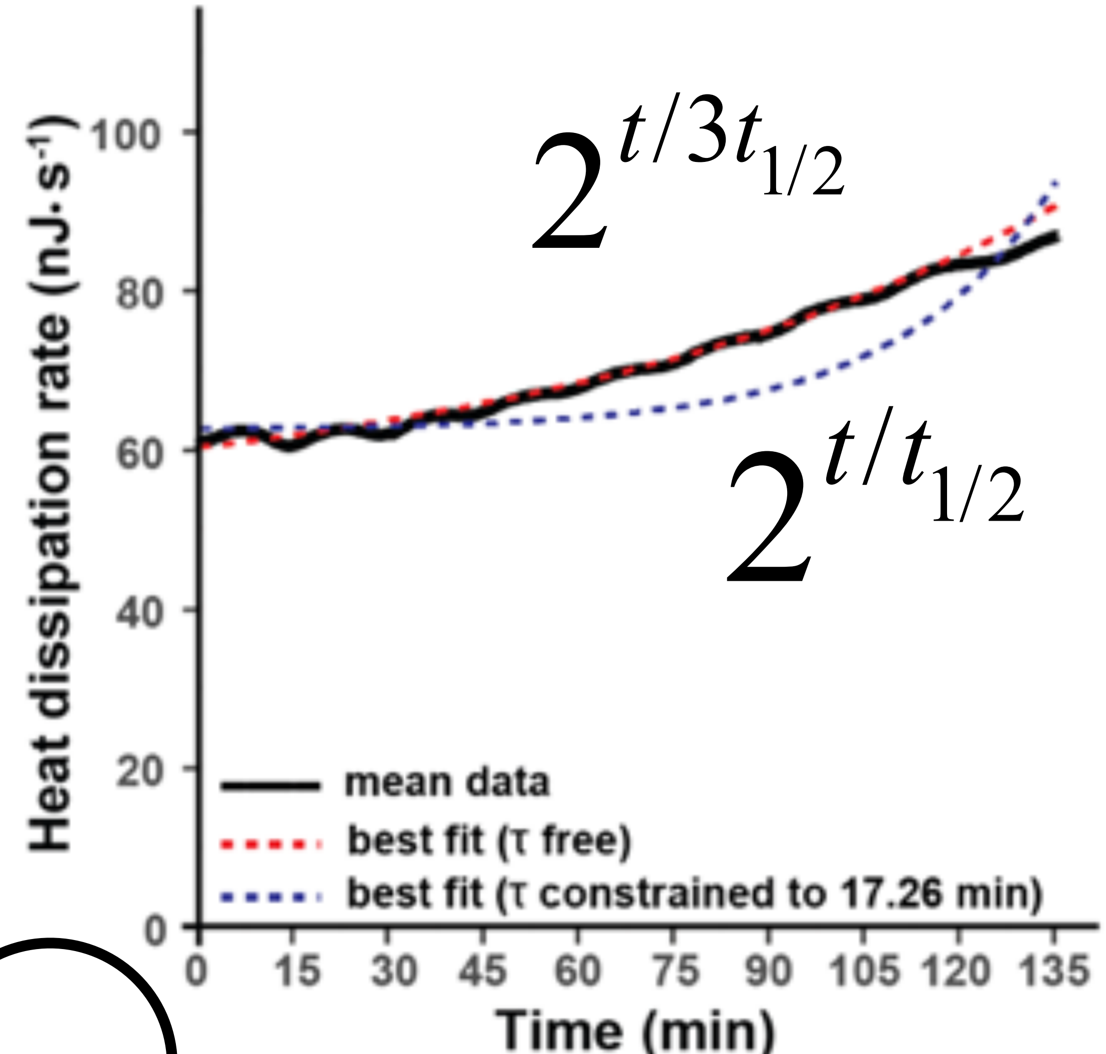
The slow component depends on proliferation:

It is blocked by nocodazole



The slow component increases "slowly" with surface area!

Number of cells N	Volume V	Surface area S
1	1	1
2	1	$2^{1/3}$
4	1	$2^{2/3}$
8	1	2



At constant volume, surface area ~ Cells$^{1/3}$

Maintenance and synthesis costs of surface area may account for the increasing trend

Table 2: Estimated energetic parameters at 28.5 °C

Parameter	Estimates		Values from fits
Volume term[1], ***A***	$90 \ \mathrm{nJ} \cdot \mathrm{s}^{-1}$		$52 \pm 12 \ \mathrm{nJ} \cdot \mathrm{s}^{-1}$
Area pre-factor[2], ***B***	β (maintenance)	γ (production)	
	β_{ATPase}[3] $= 0.24 \cdot 10^{-3} \ \mathrm{pJ} \cdot \mathrm{s}^{-1} \cdot \mu\mathrm{m}^{-2}$	γ_{lipid}[4] $= 0.12$ to 24 * $\mathrm{pJ} \cdot \mu\mathrm{m}^{-2}$	
	$\beta_{\mathrm{turnover}}$[5] $= 0.02 \cdot 10^{-3} \ \mathrm{pJ} \cdot \mathrm{s}^{-1} \cdot \mu\mathrm{m}^{-2}$	$\gamma_{\mathrm{protein}}$[6] $= 4.3$ to 128* $\mathrm{pJ} \cdot \mu\mathrm{m}^{-2}$	
	$B = \left(\sum \beta_{\mathrm{i}} + \frac{\ln(2)}{3T} \sum \gamma_{\mathrm{i}}\right) \cdot S_1 = 0.9$ to $32 \ \mathrm{nJ} \cdot \mathrm{s}^{-1}$* (see[7],[8])		$8.2 \pm 3.2 \ \mathrm{nJ} \cdot \mathrm{s}^{-1}$

Production costs from Lynch & Marinov 2015, 2017

Maintenance costs from membrane ATPases (e.g. ion pumps)

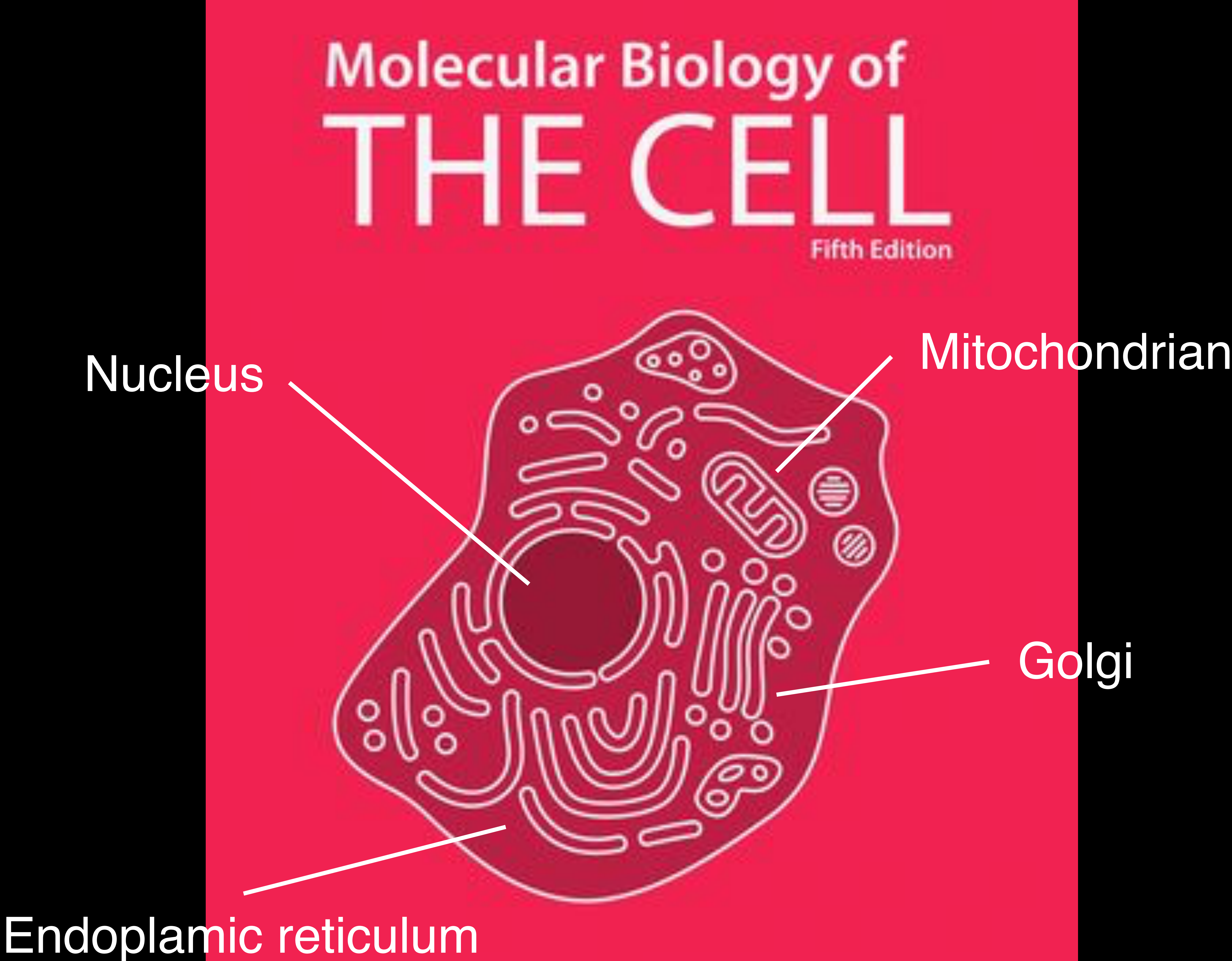
Summary of heat dissipation in early embryogenesis

1. Initial level: basal metabolic rate
2. Oscillations: cell cycle oscillator
3. Slow increase: surface area increase

Two energetic stories

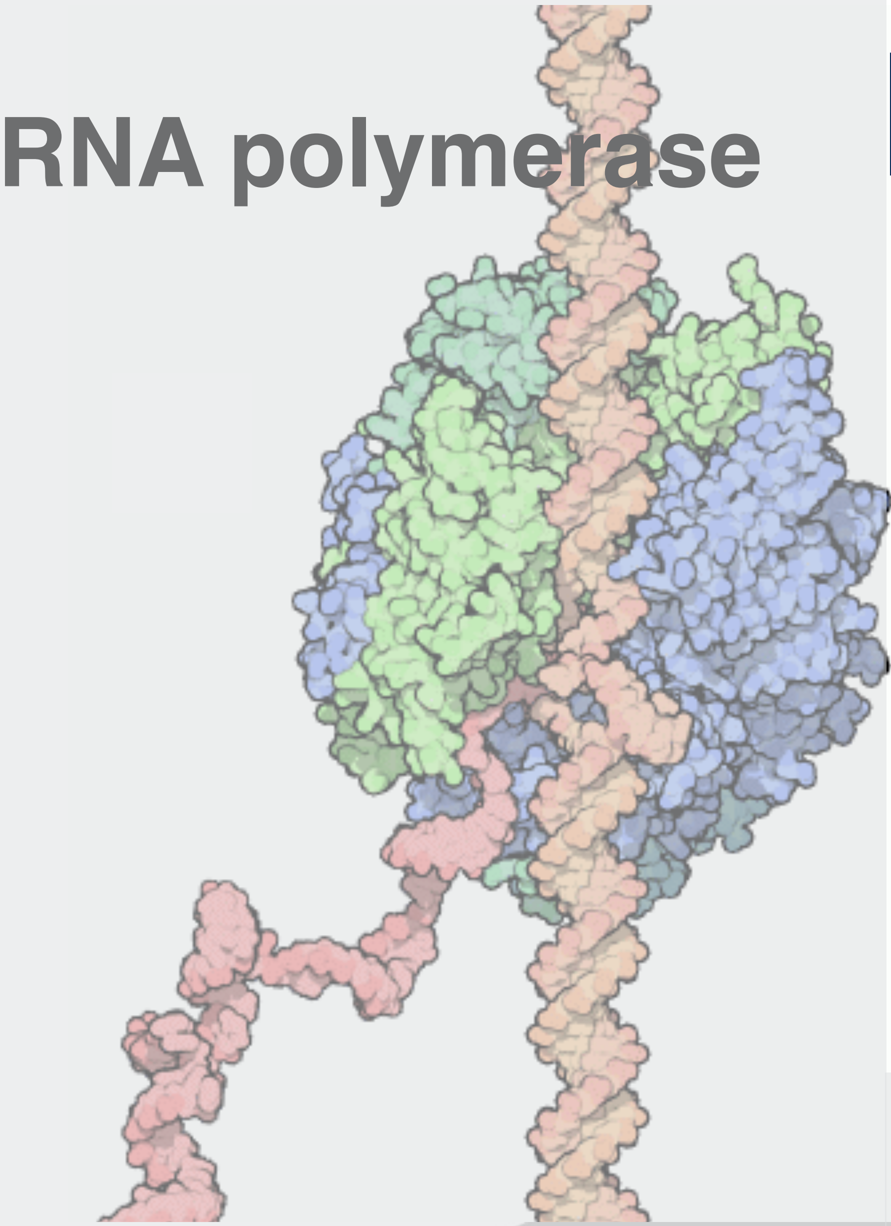
1. Early development in zebrafish

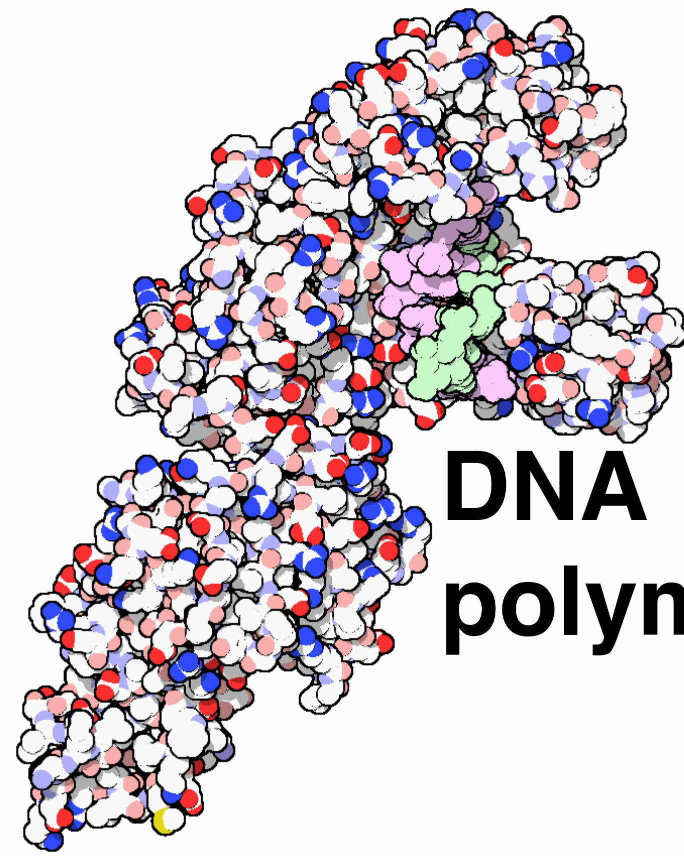
2. Molecular machines

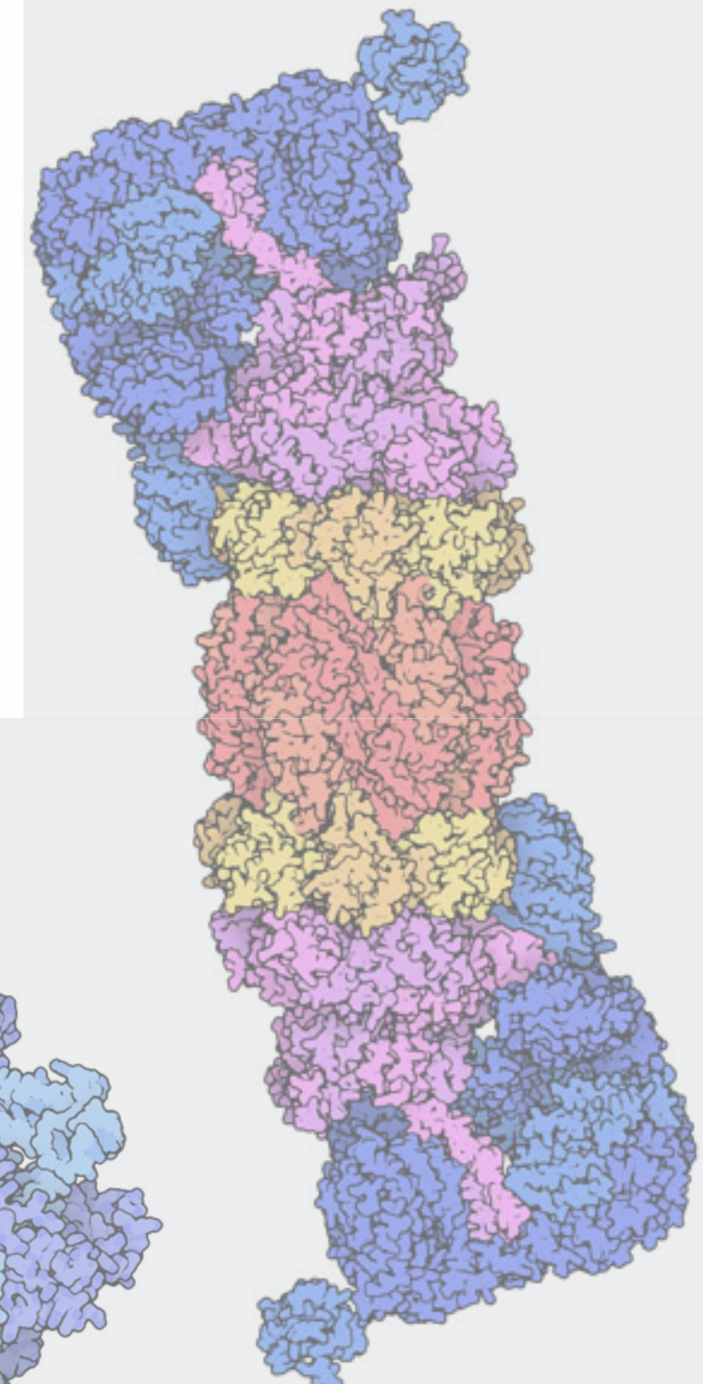


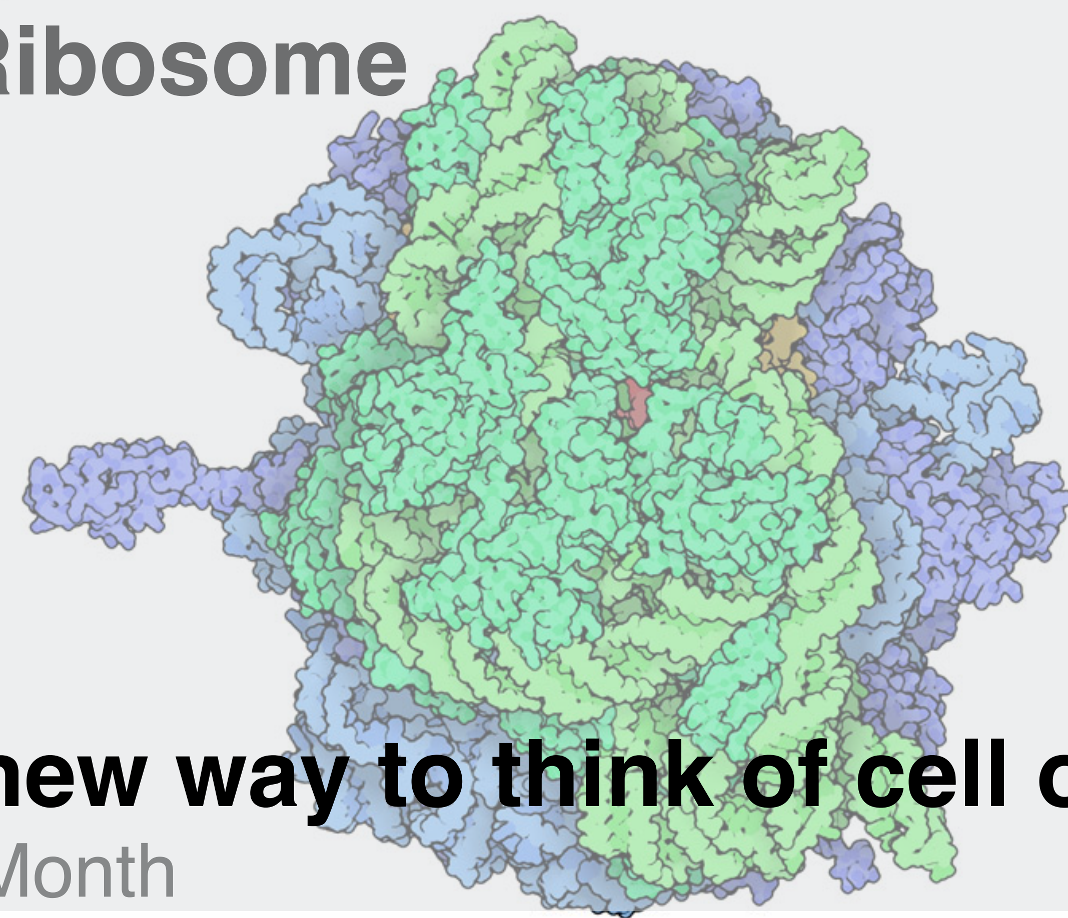
Organelles: the traditional way to think of cell organisation

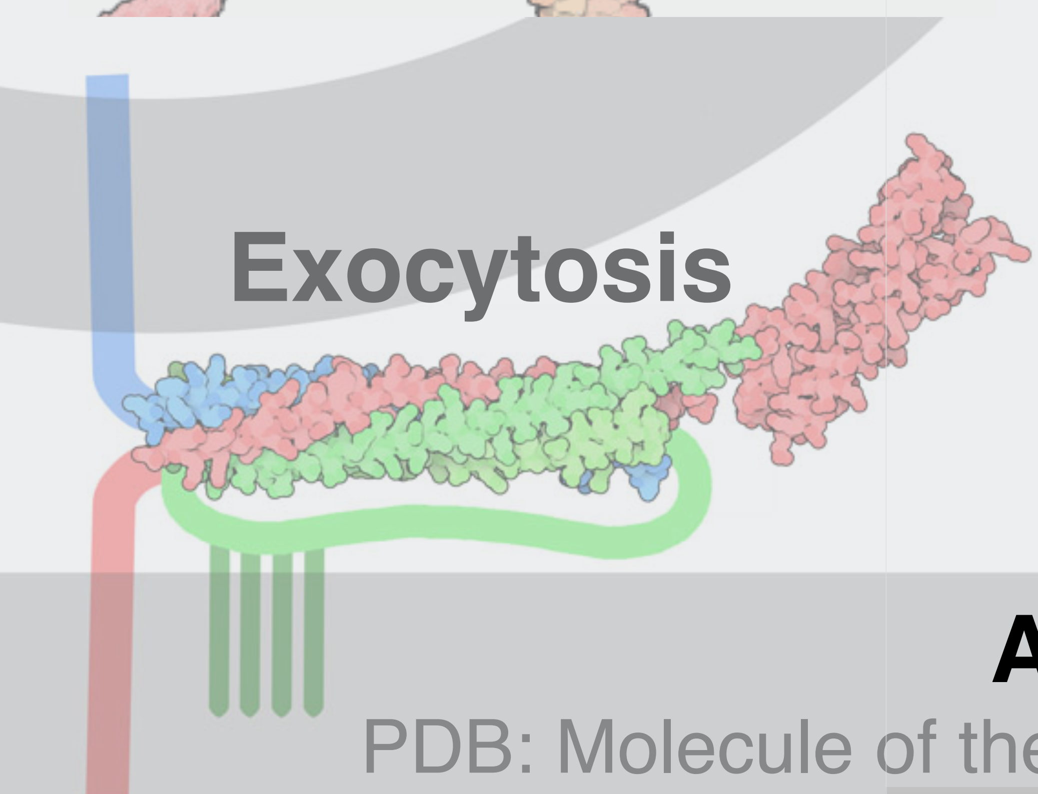
Molecular machines









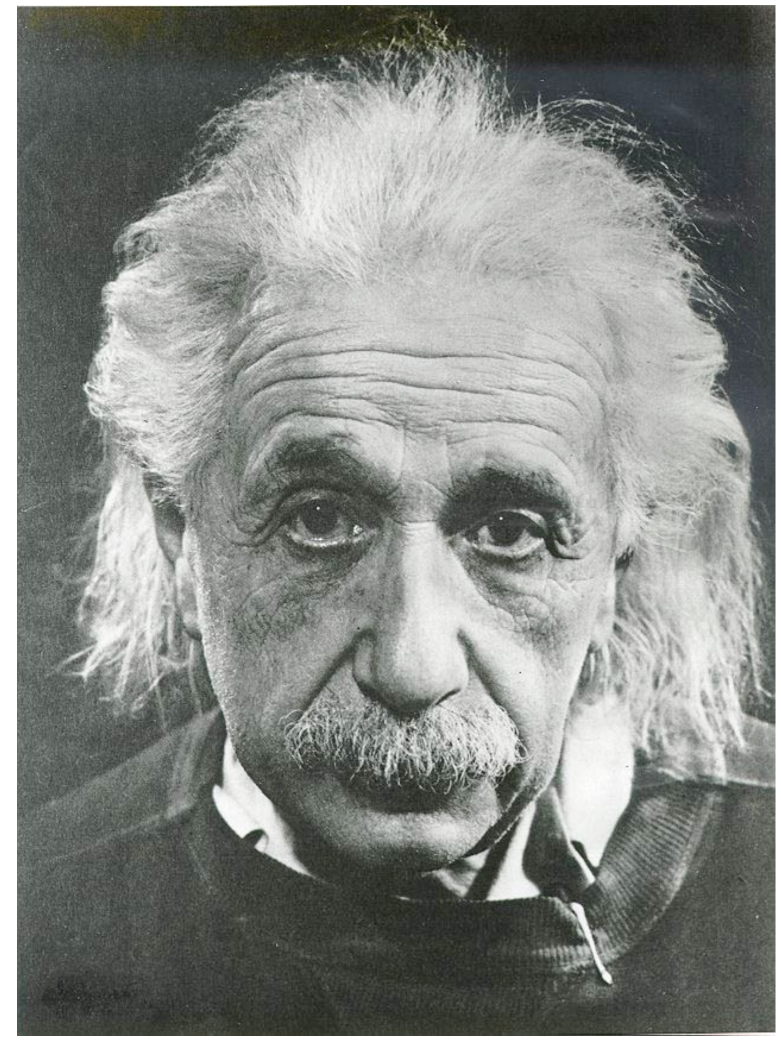


A new way to think of cell organisation

PDB: Molecule of the Month

We make astronomical amounts of DNA!

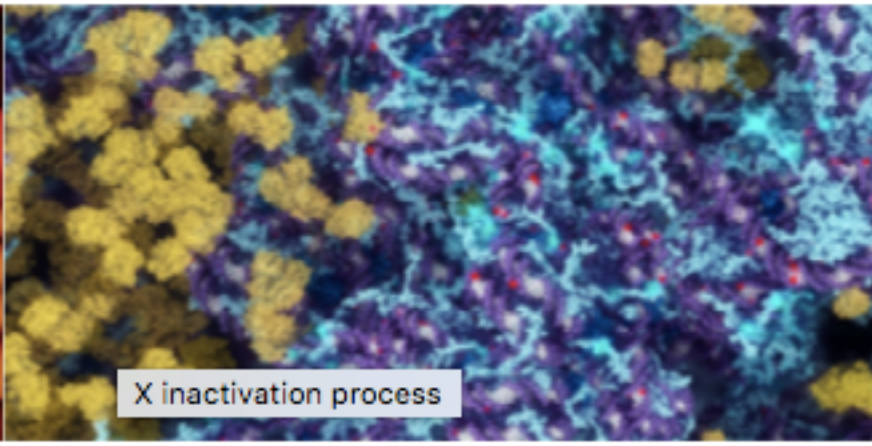
Einstein young



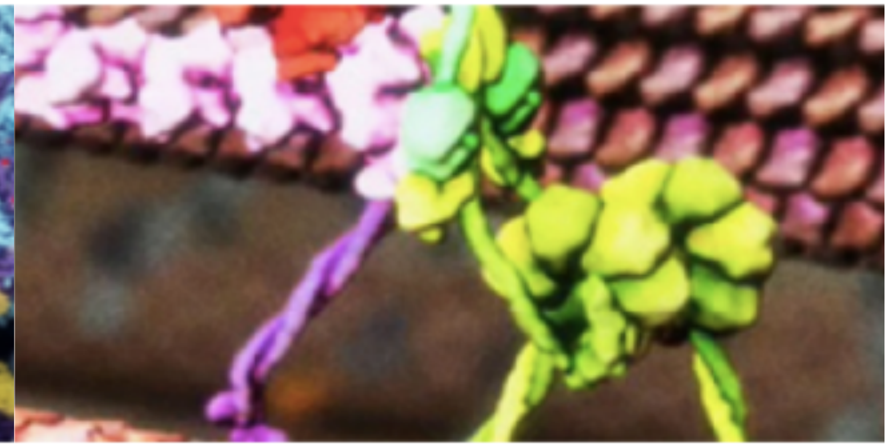
Einstein old

≈1 light year of DNA is synthesized and segregated during the life of a 70-year old (≈10,000,000,000,000 kilometers)

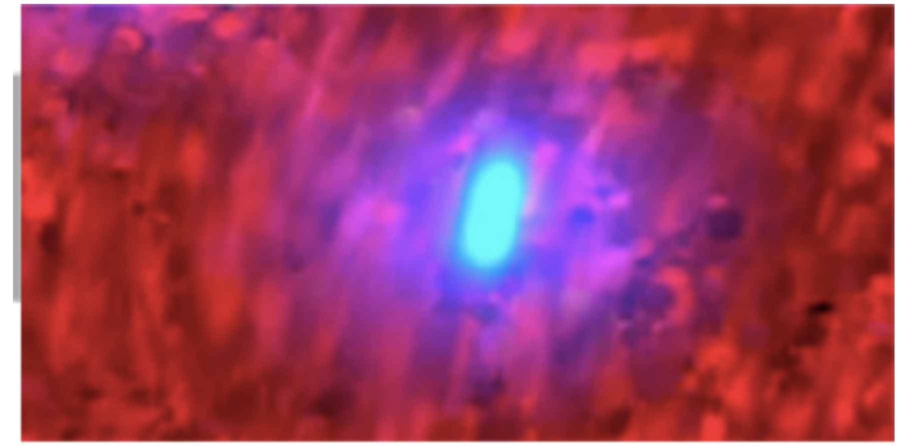
John Diffley



X Inactivation and Epigenetics



Chromosome and Kinetochore



Haemoglobin and Sickle Cell Anaemia

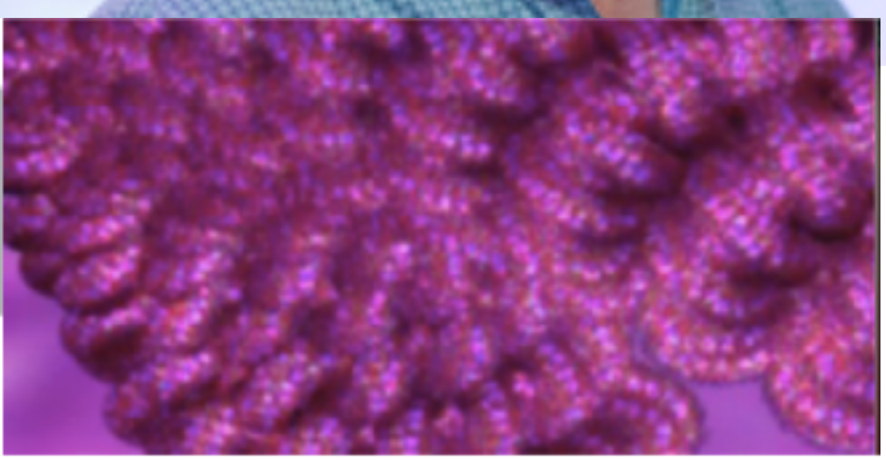
Drew Berry

Cell Animator

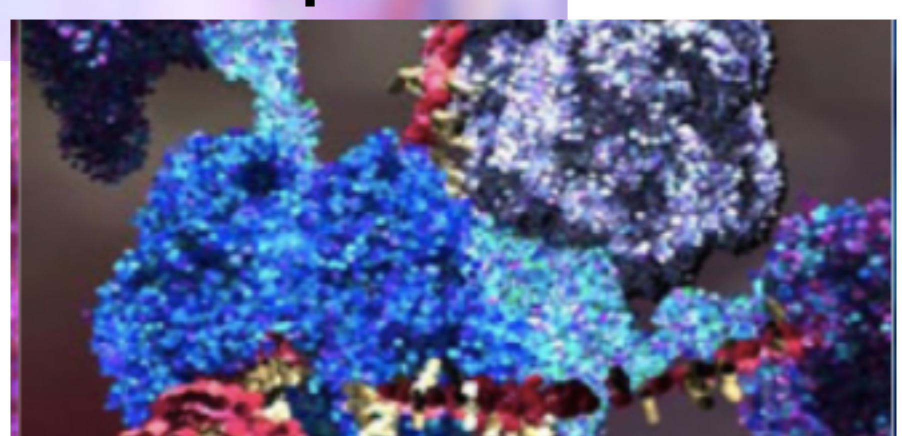
Walter and Eliza Hall Insitute

Melbourne, Australia

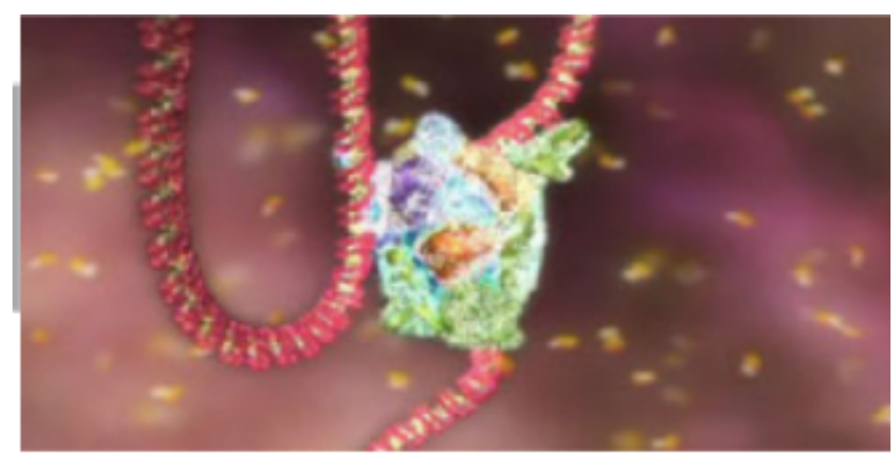
http://www.wehi.edu.au/wehi-tv/wehitv



DNA Central Dogma Part 1 - Transcription

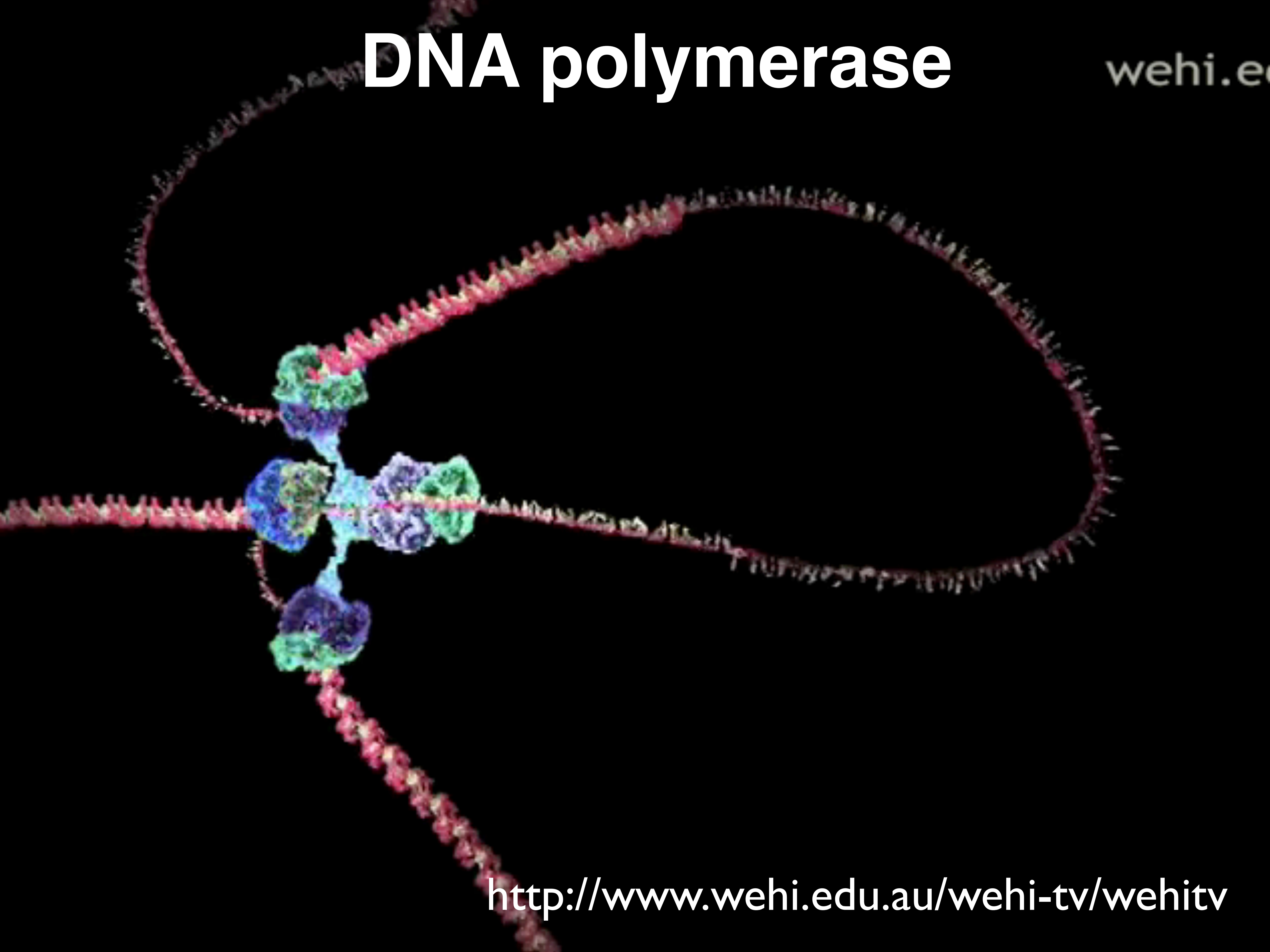


DNA Central Dogma Part 2 - Translation



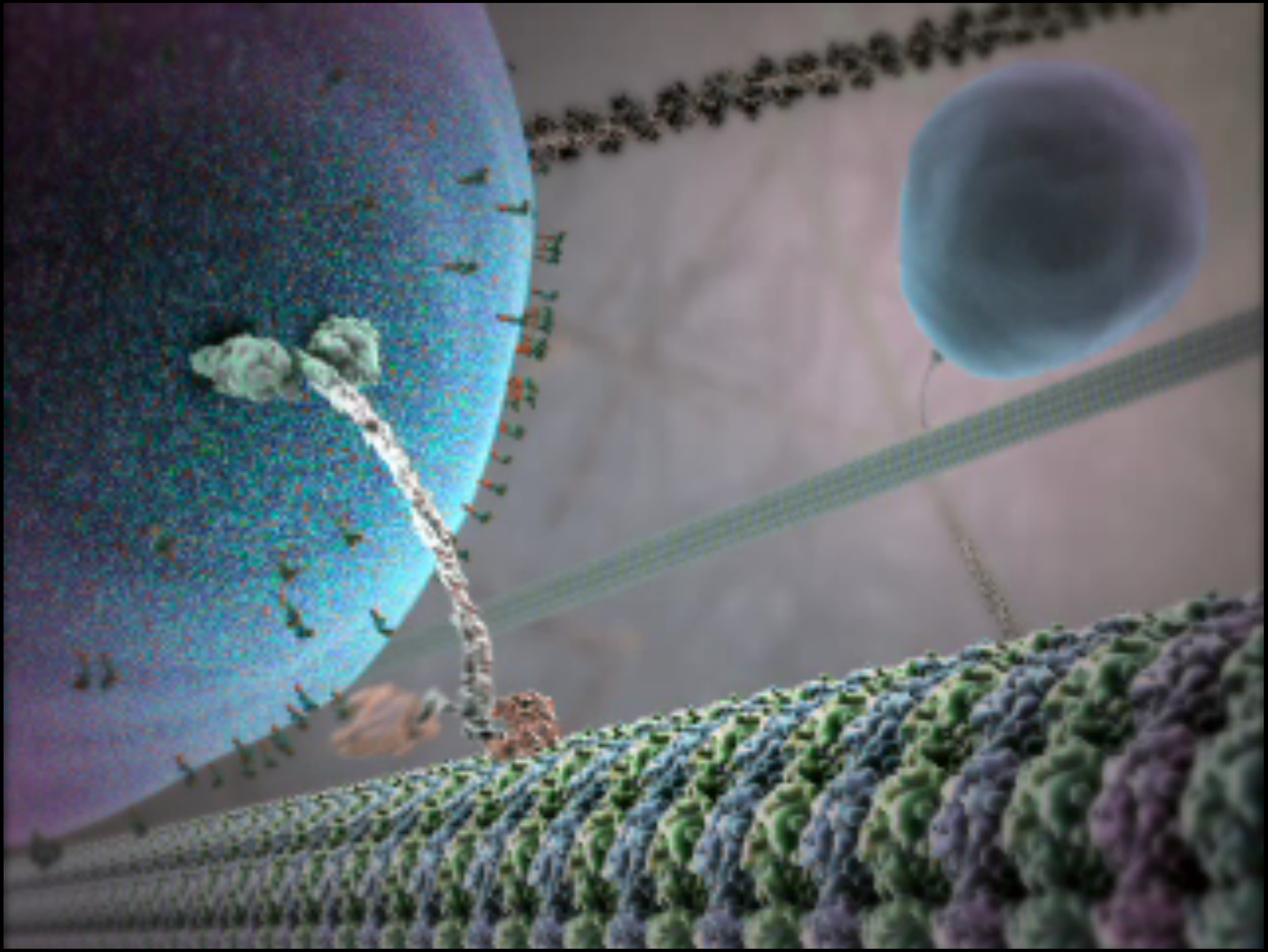
Molecular Visualisations of DNA

DNA polymerase



http://www.wehi.edu.au/wehi-tv/wehitv

My favorite molecular machine: kinesin



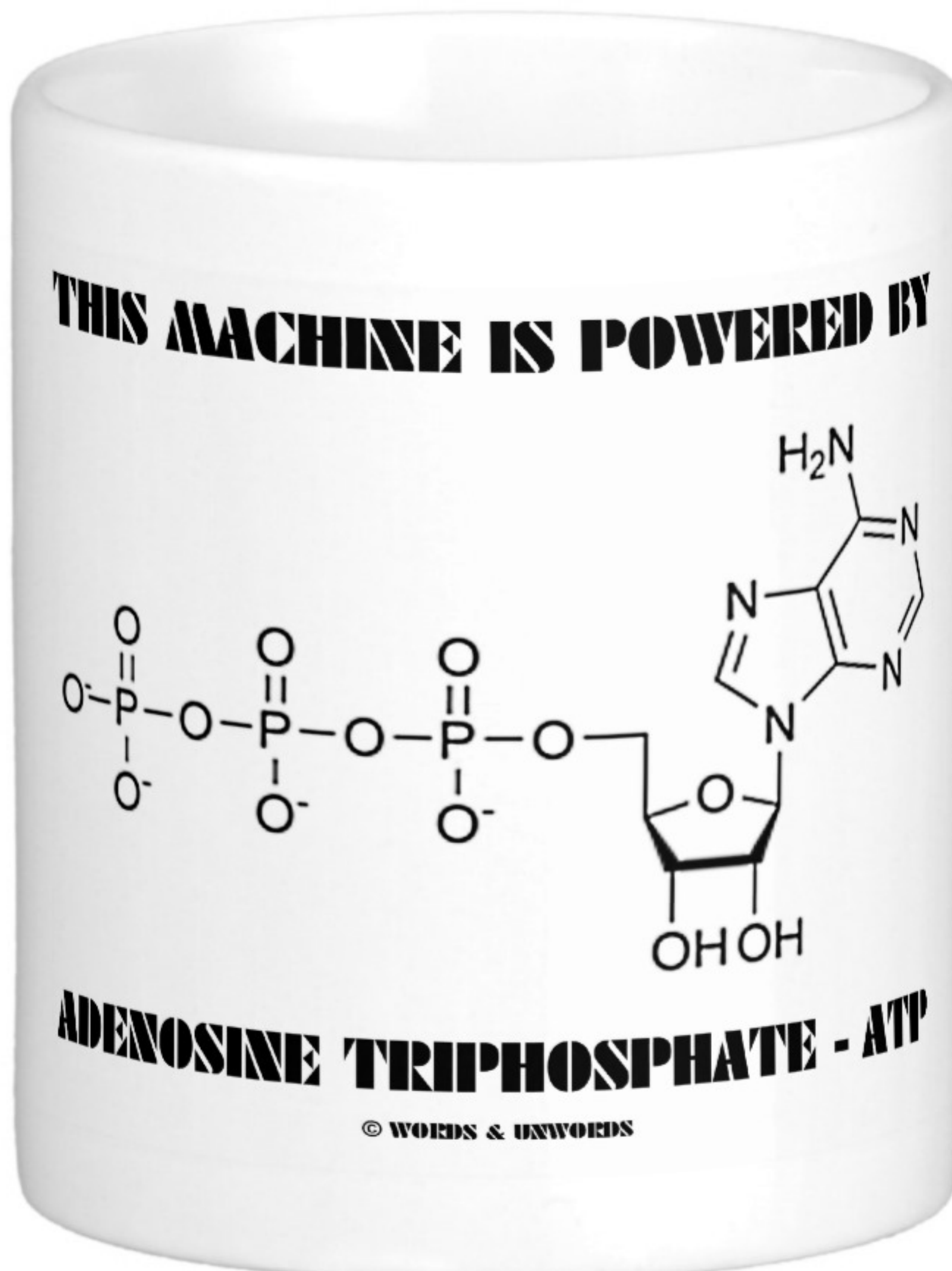
The inner life of the cell (Harvard Univ.)

What are molecular machines?

Molecular machines are enzymes which convert **chemical energy** (usually in the form of ATP) into **mechanical work** (protein conformational changes) that is used to power motility, synthesis (DNA, RNA, sugars, polymers), membrane fusion, **signaling**, etc. etc.

Molecular machines differ from man-made engines that use heat or elecric fields as an intermediate between chemical energy (e.g. oil) and mechanical work

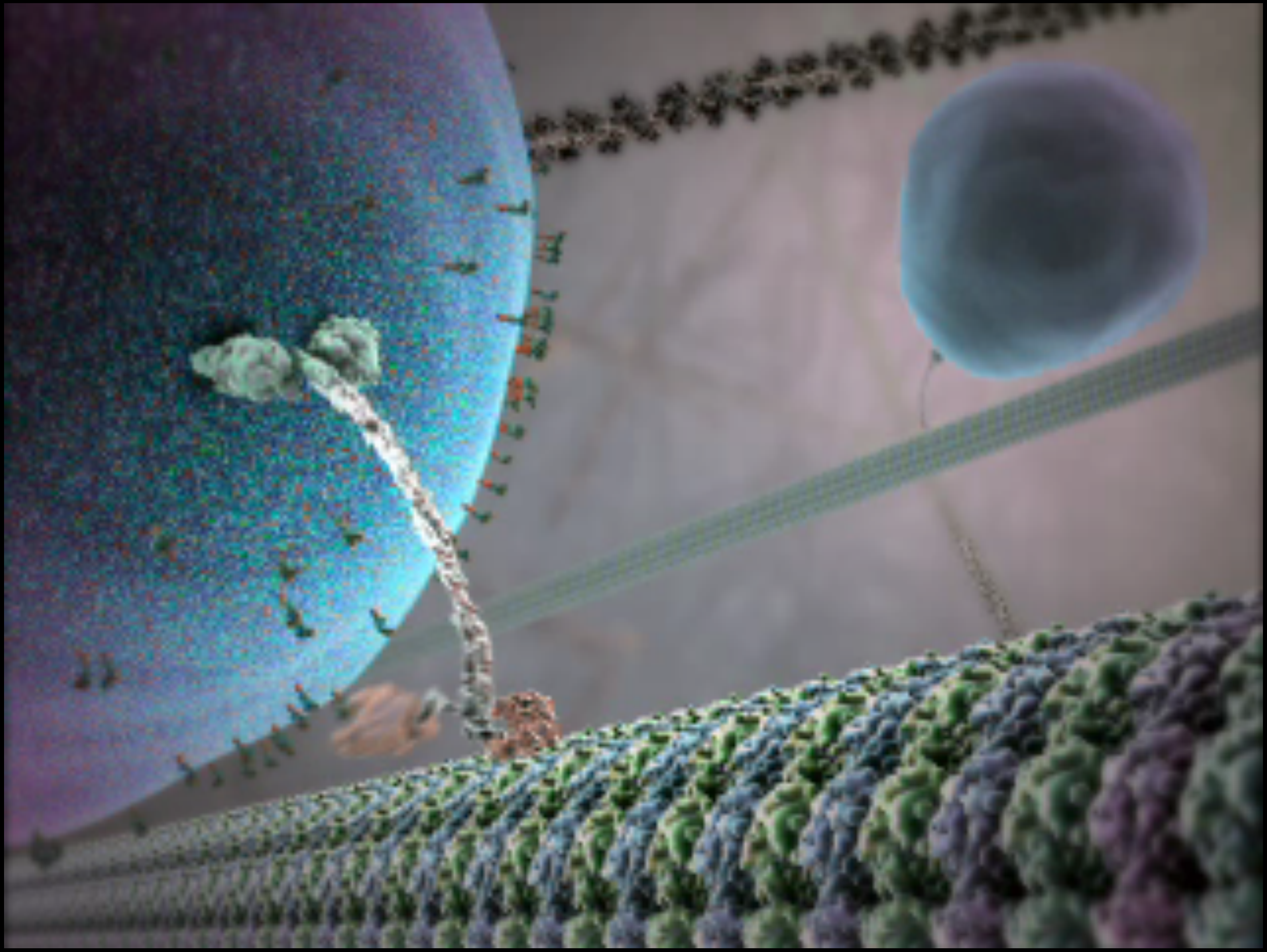
ATP is the universal energy currency in cells



MW ≈ 500

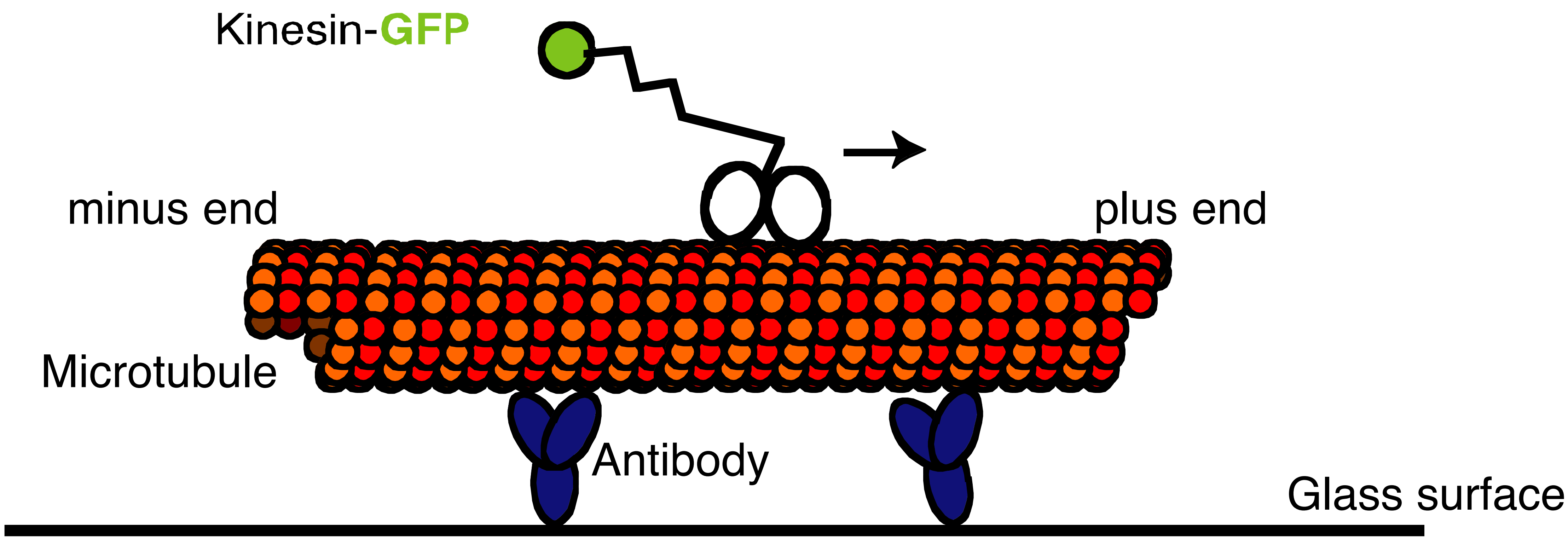
We consume our body weight of ATP every day!

Fact or fiction?



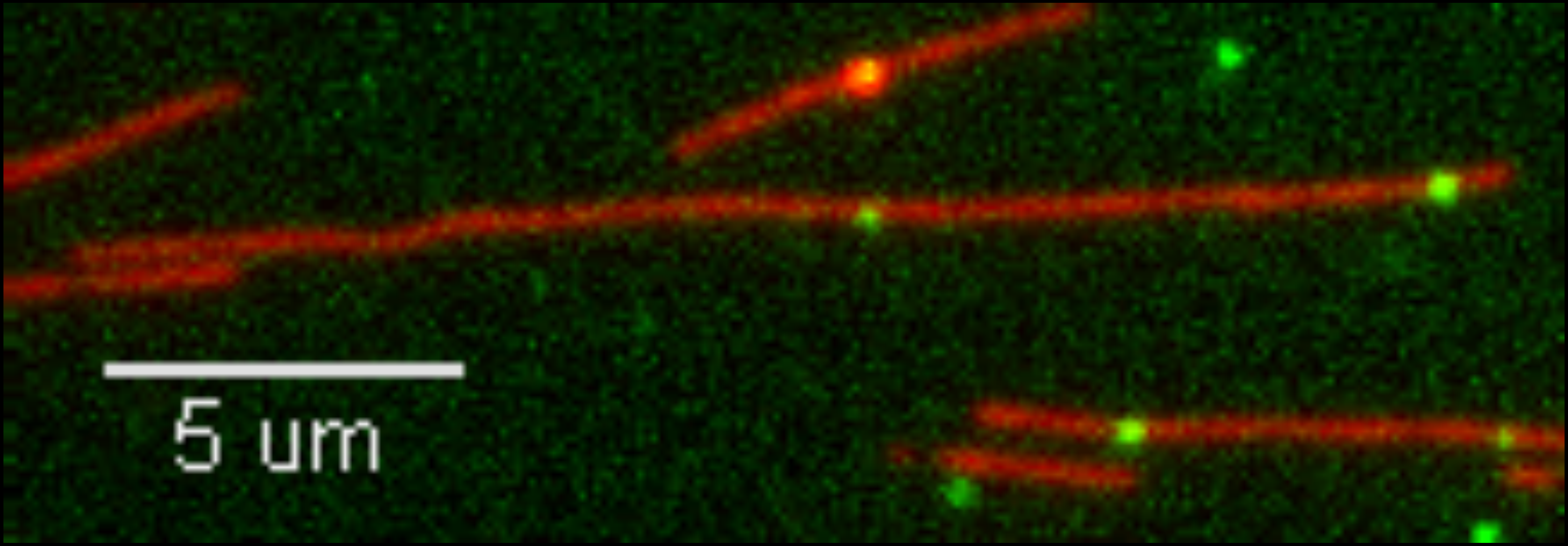
The inner life of the cell (Harvard Univ.)

Single-molecule fluorescence assay



with Stefan Diez

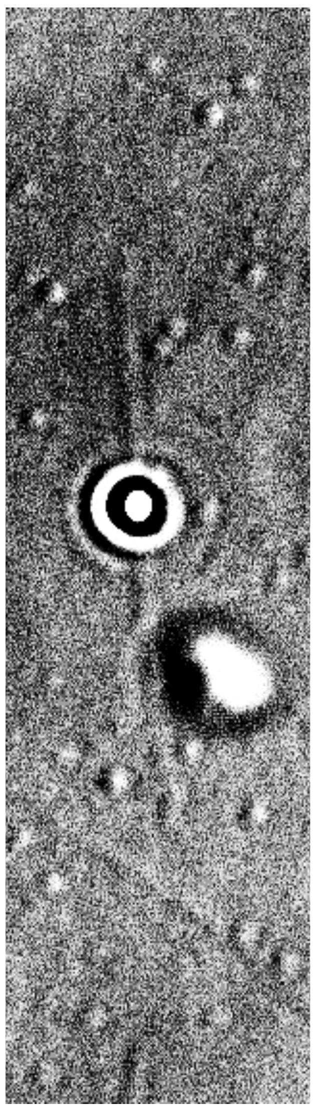
Kinesin is "processive": one molecule takes hundreds of steps

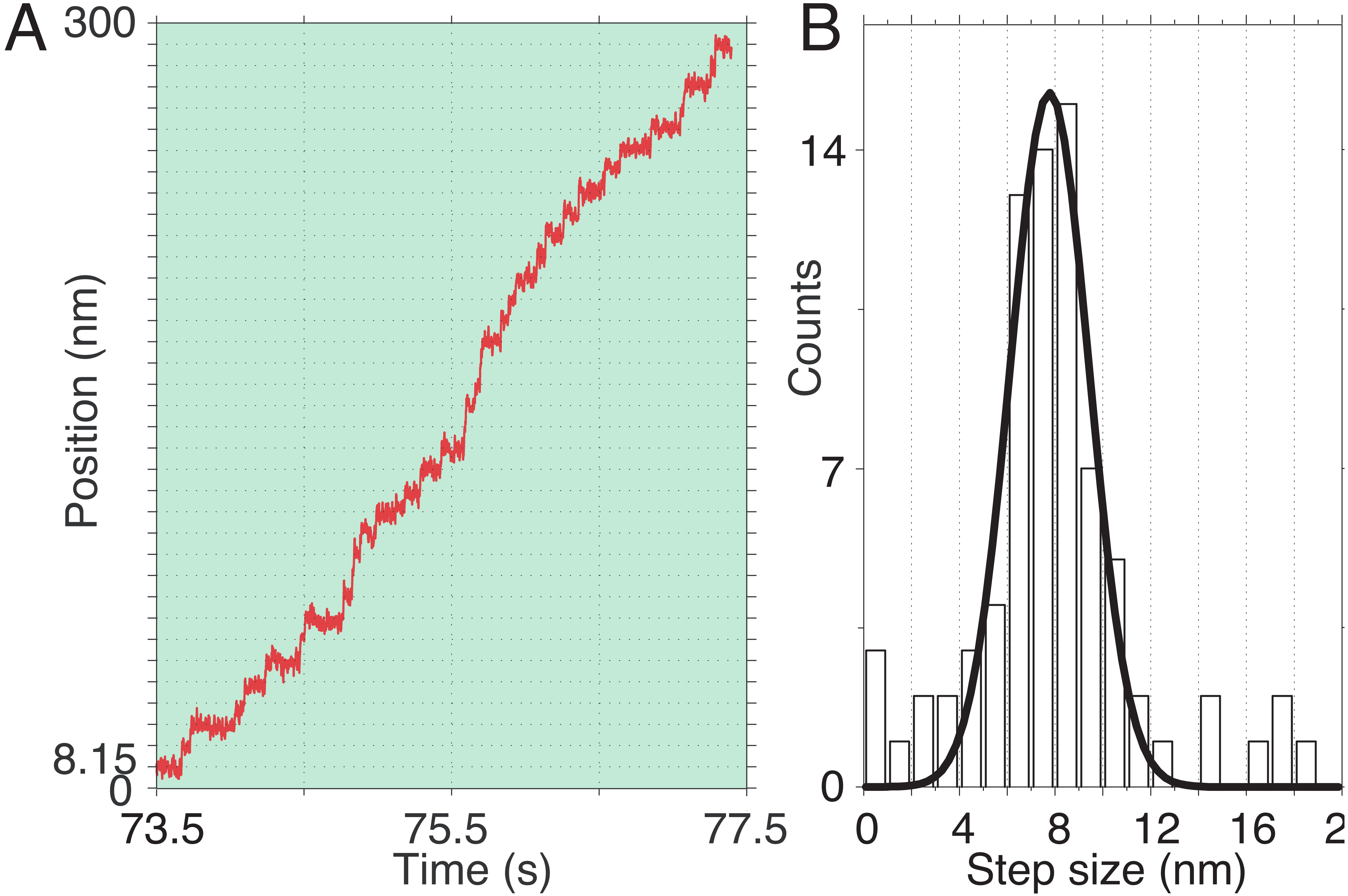


+ATP (the chemical fuel)

Stefan Diez

Kinesin takes 8-nm steps

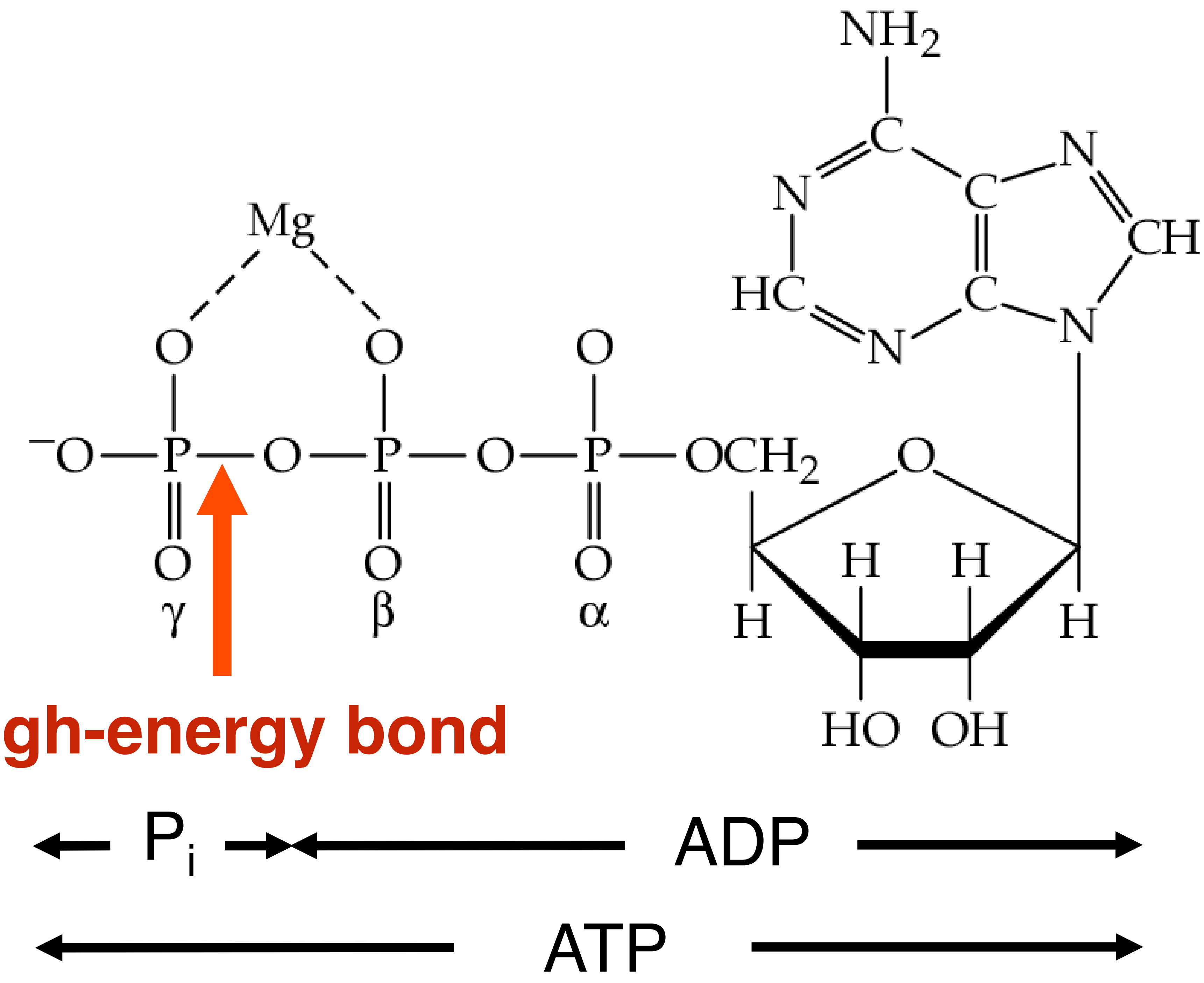




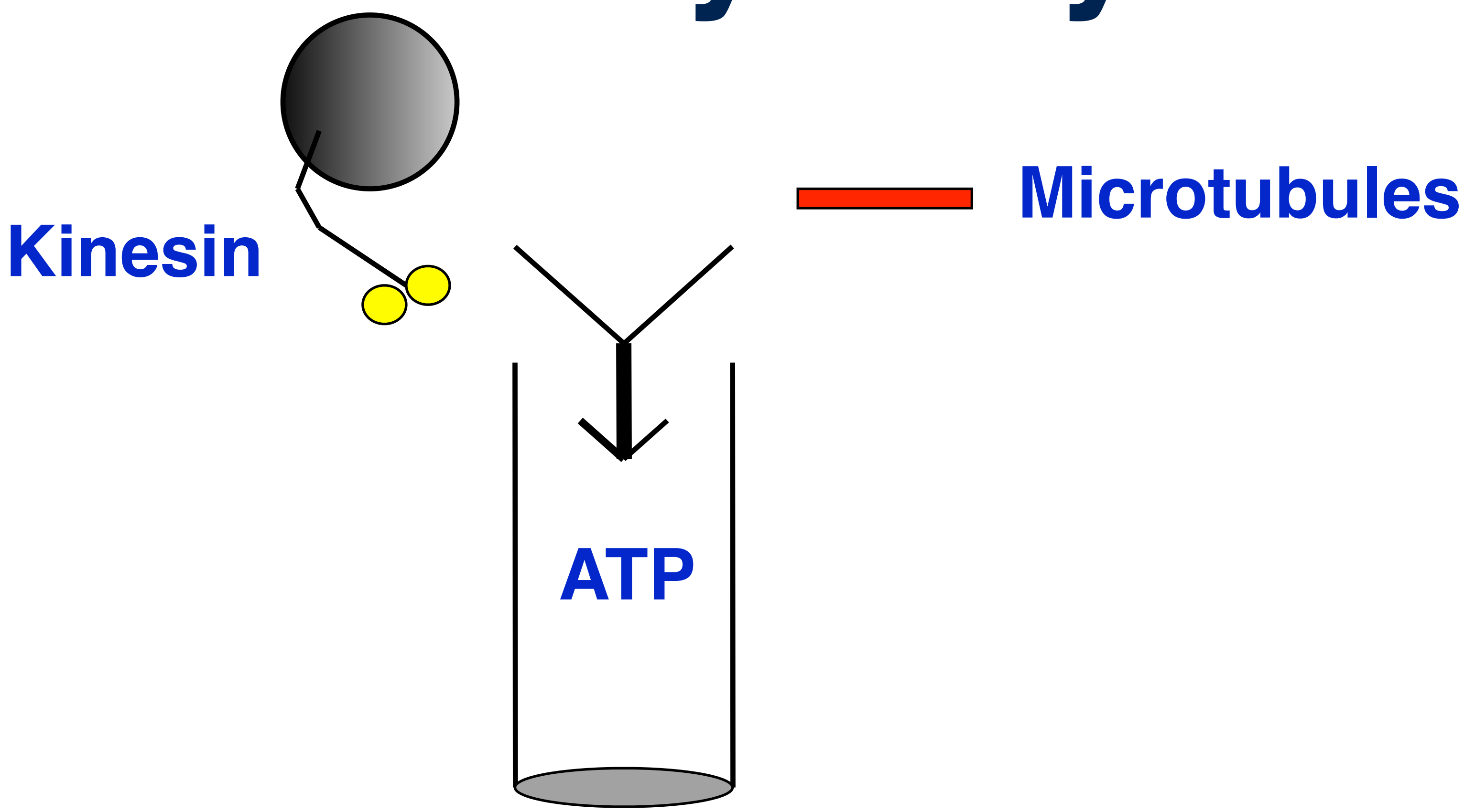
Volker Bormuth

How many ATPs per step?

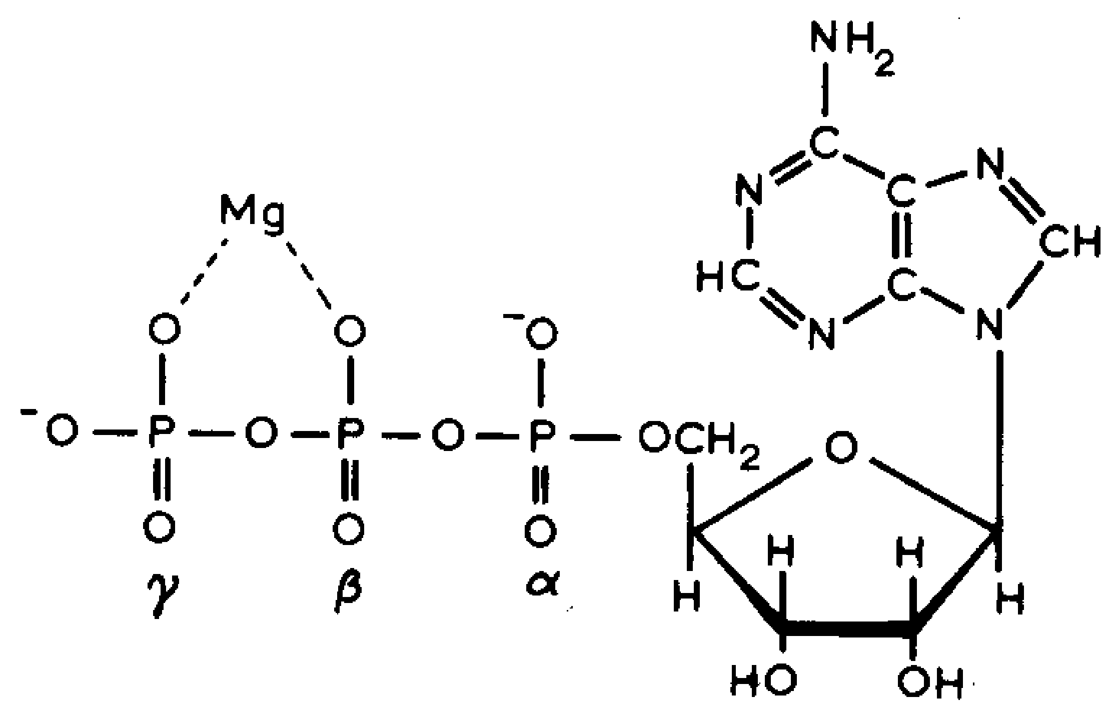
ATP hydrolysis is required for motility



Measuring the ATP hydrolysis rate

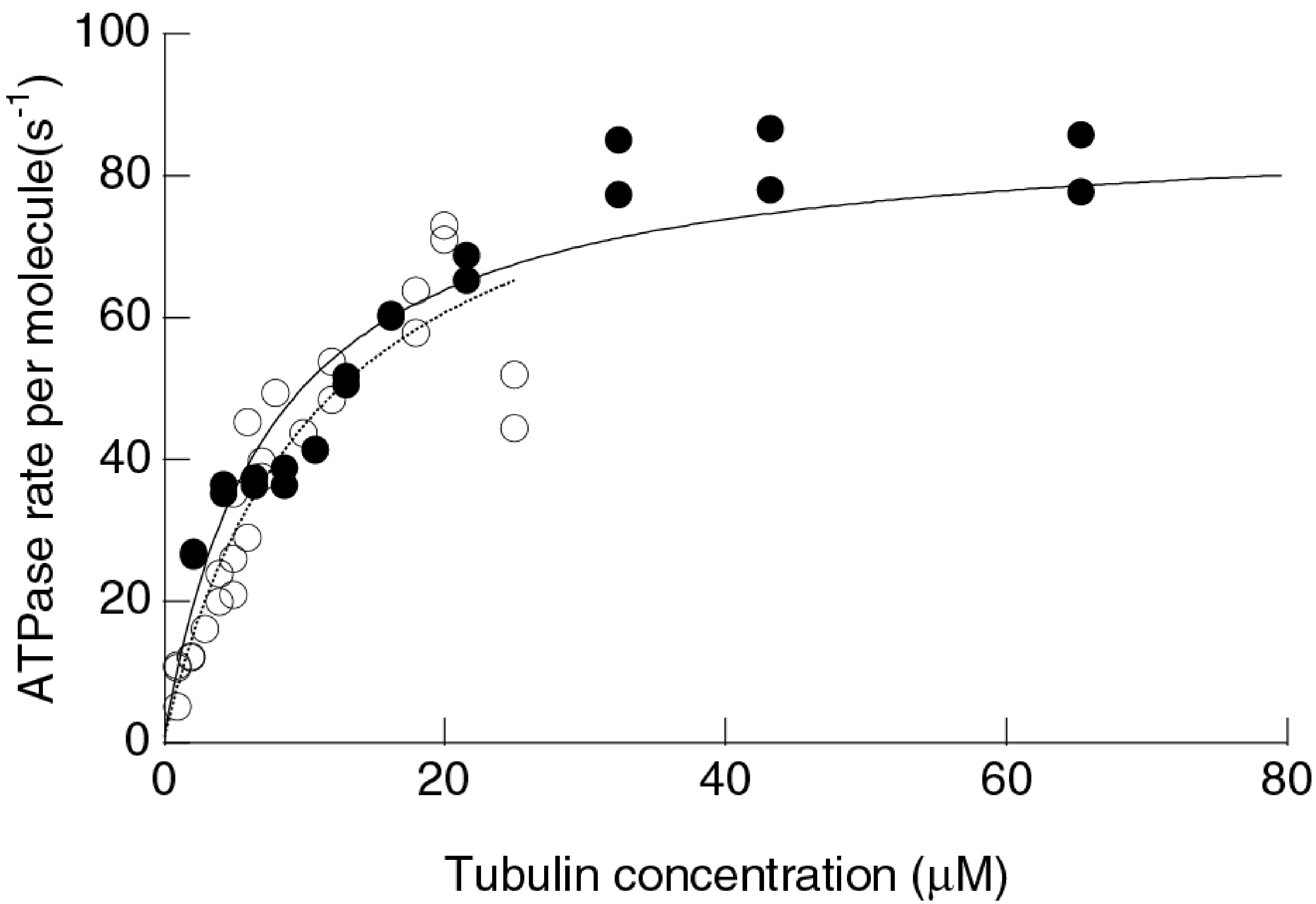


ATP → ADP + P_i



Dye that changes color when phosphate binds (e.g. malachite green). Many other ways.

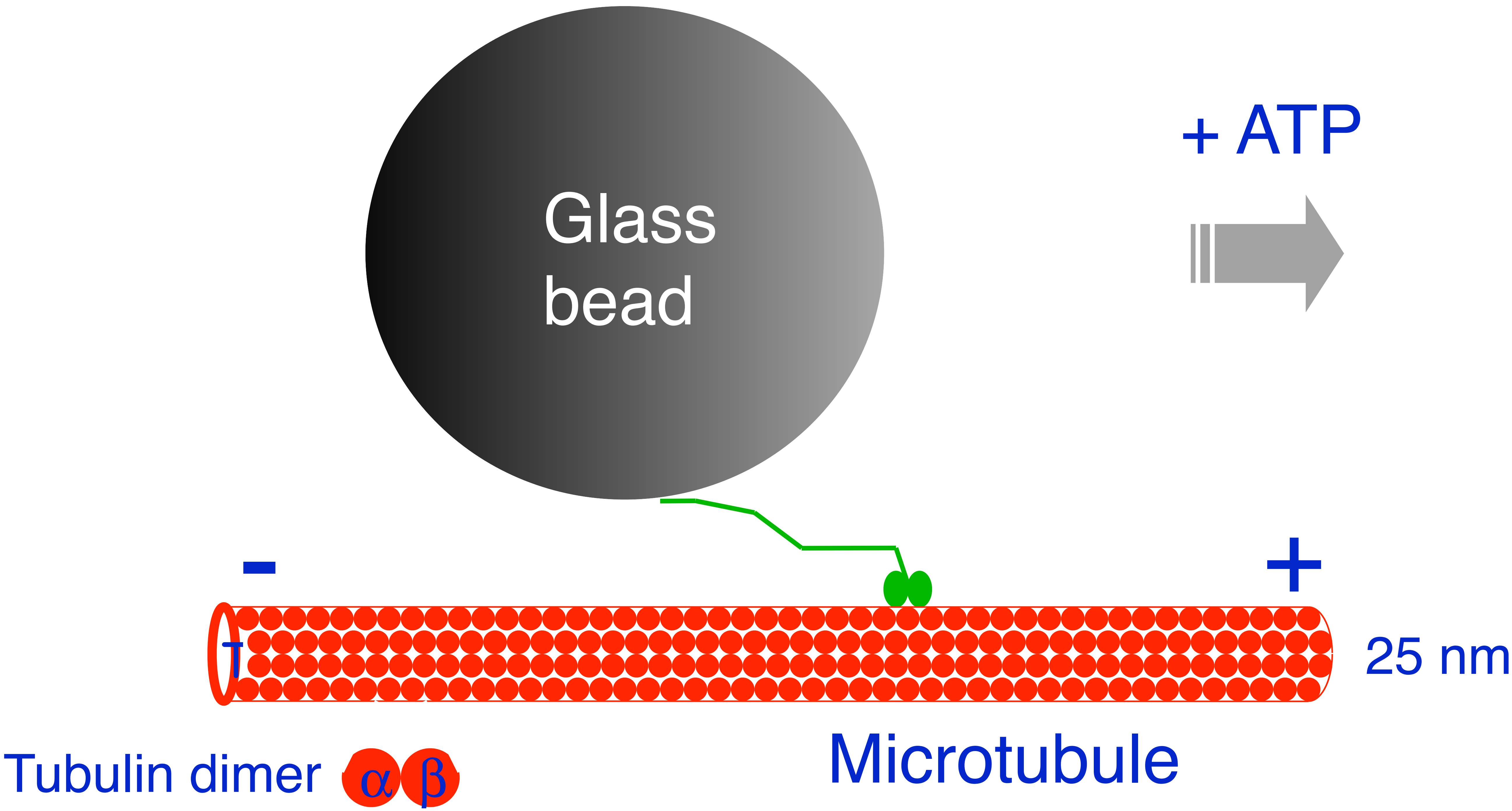
Microtubule-Stimulated ATPase Rates of Single Kinesin Molecules Attached to Beads



Measure the speed under identical conditions

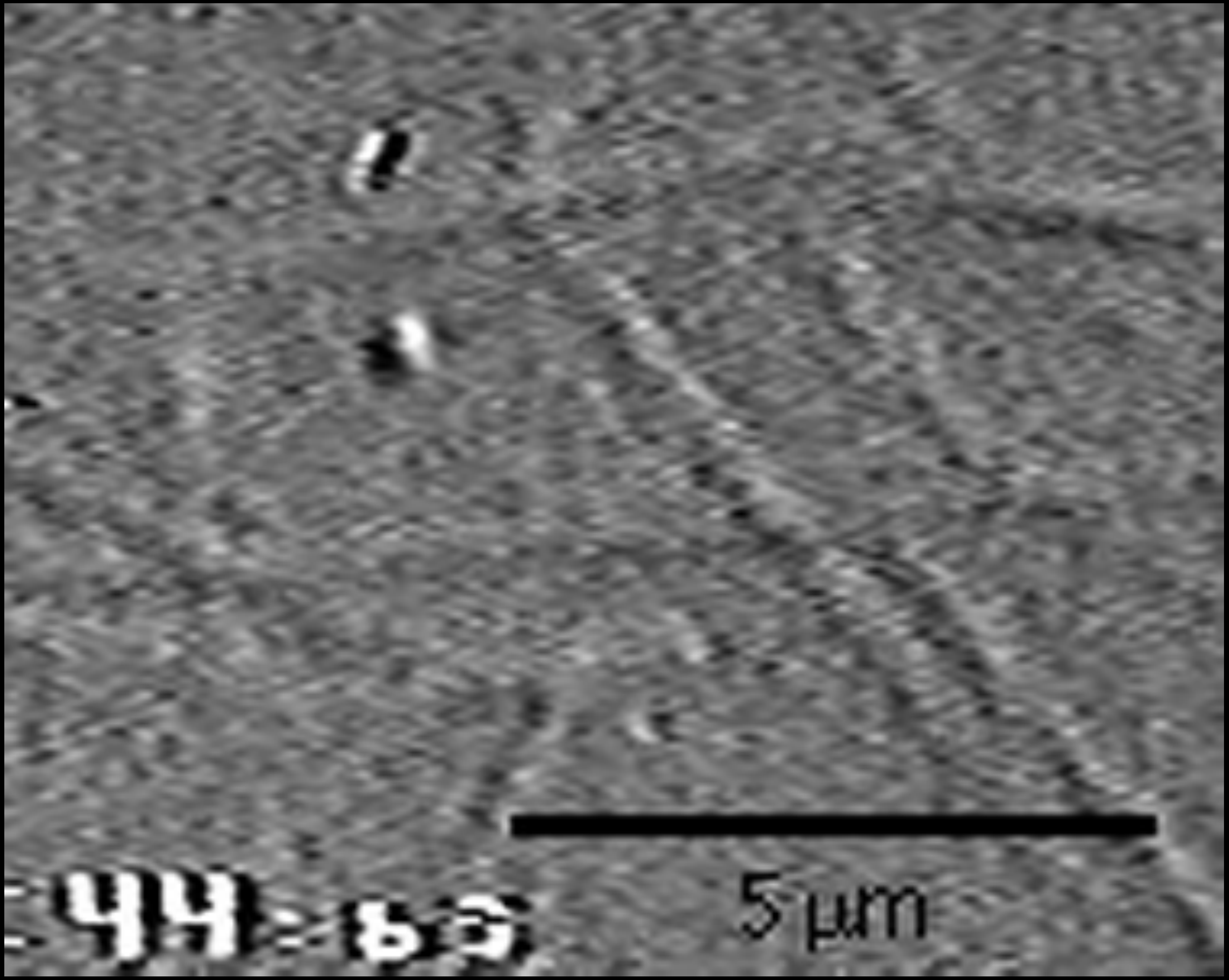
Coy et al. 1999

Bead assay



David Coy

Bead assay with purified kinesin



real time

Speed ≈ 800 nm/s

David Coy

Kinesin takes one 8-nm step per ATP hydrolyzed

Speed ±SE (nm/s)	ATPase ±SE (s^{-1})	Distance/ATP (nm/ATP)	Stoichiometry (steps/ATP)
772 ± 29	88 ± 6	8.7 ± 0.7	1.08 ± 0.09

Coy et al. 1999

Mechanics of the kinesin motor

Single molecules move processively (up to 1000 nm)
8 nm steps (between tubulin dimers)
1 ATP hydrolyzed per step

Maximum velocity:	800 nm/s	(plenty enough to move a vesicle)
Maximum force:	6 pN	
Maximum work:	48 pN.nm (=6 pN x 8 nm)	
Energy from ATP hydrolysis	100 pN.nm (at cell conc.) (~25 *kT*)	
Maximum efficiency:	~50%	

But how does it move?

Kinesin superfamily of motor proteins

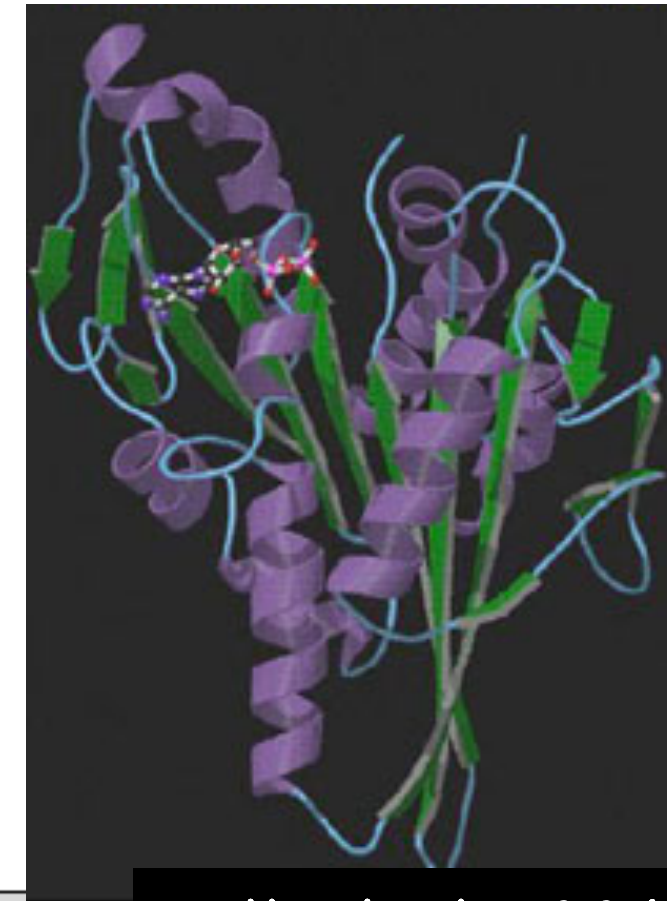
Kull et al. 1996

Humans have 41 different kinesis!!

Mitosis and meiosis Organelle transport

Lawrence et al. 2002

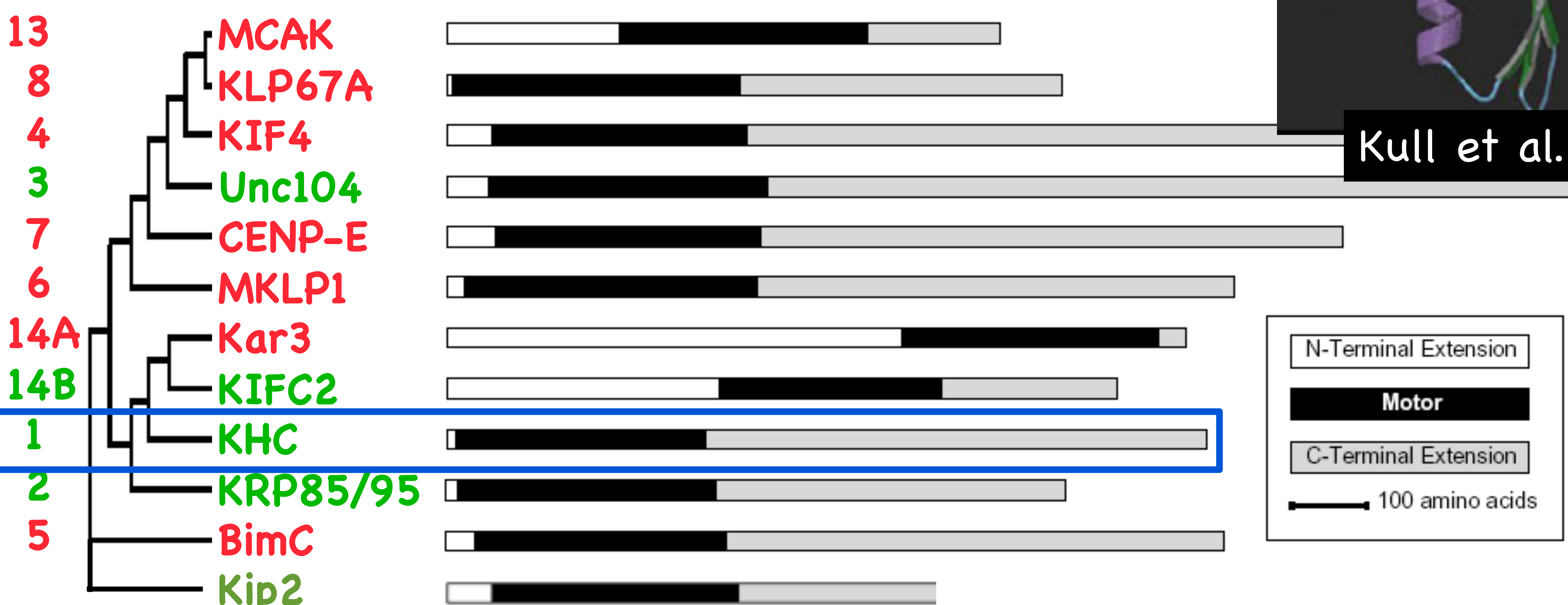
Transport kinesins



Kull et al. 1996

Subfamily names

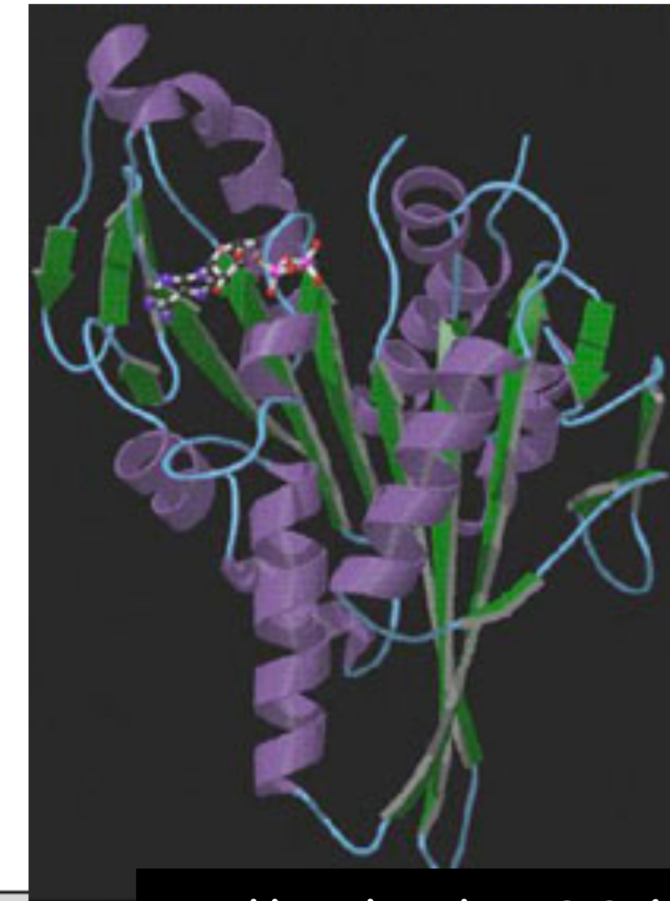
New **Old**



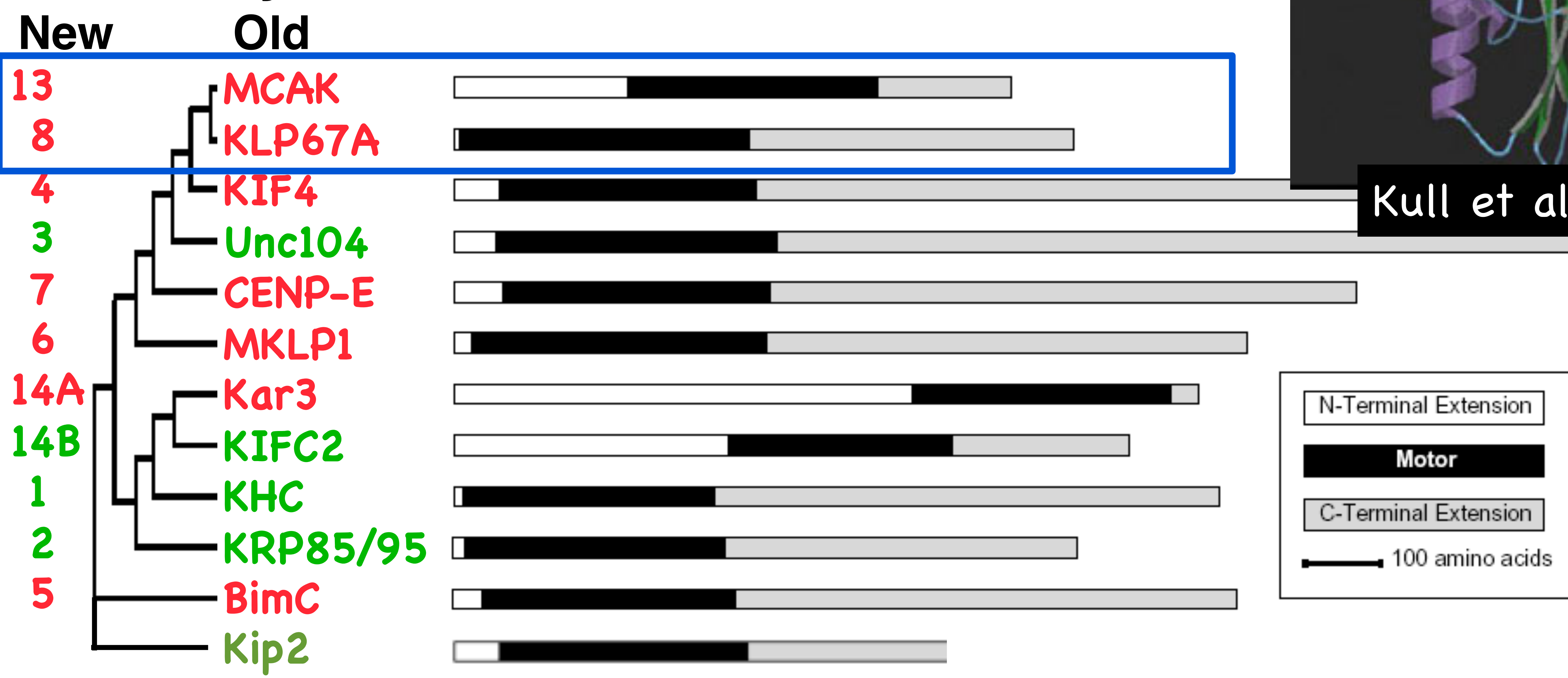
Mitosis and meiosis Organelle transport

Lawrence et al. 2002

Depolymerizing kinesins


Kull et al. 1996

Subfamily names

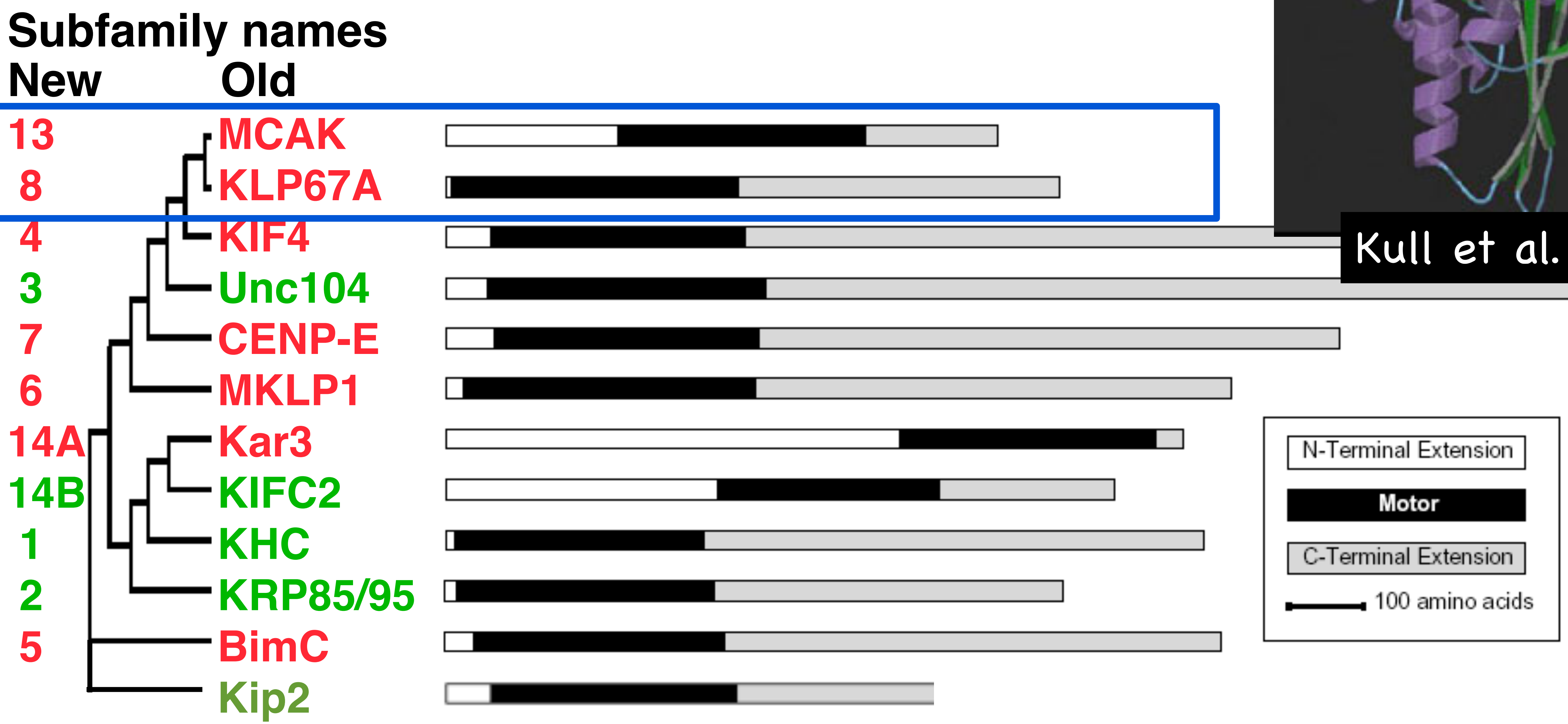


Mitosis and meiosis Organelle transport

Lawrence et al. 2002

Depolymerizing kinesins

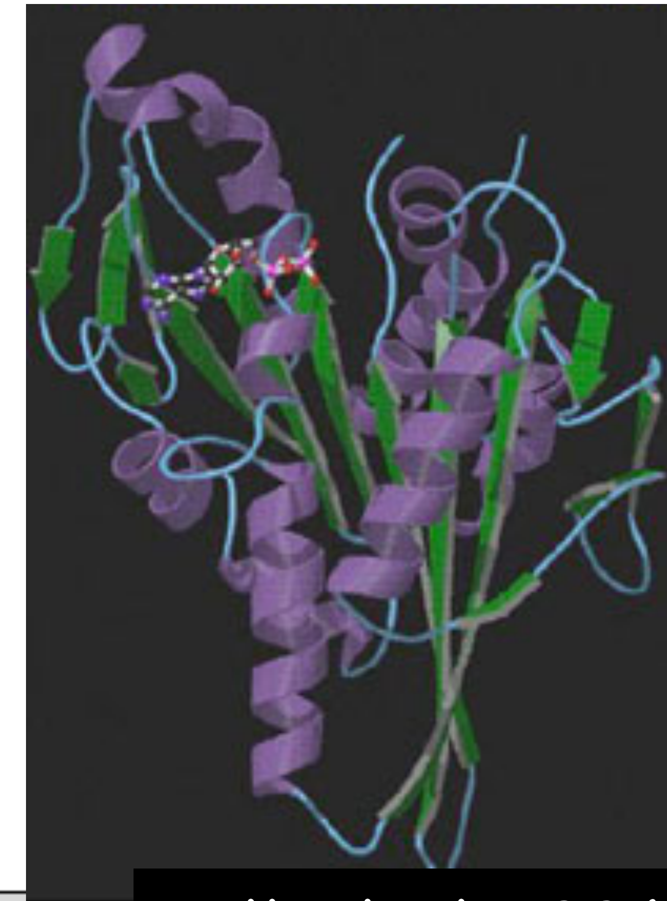
Kull et al. 1996



Mitosis and meiosis Organelle transport

Lawrence et al. 2002

Kinesin-8
(depolymerizing kinesin)



Kull et al. 1996

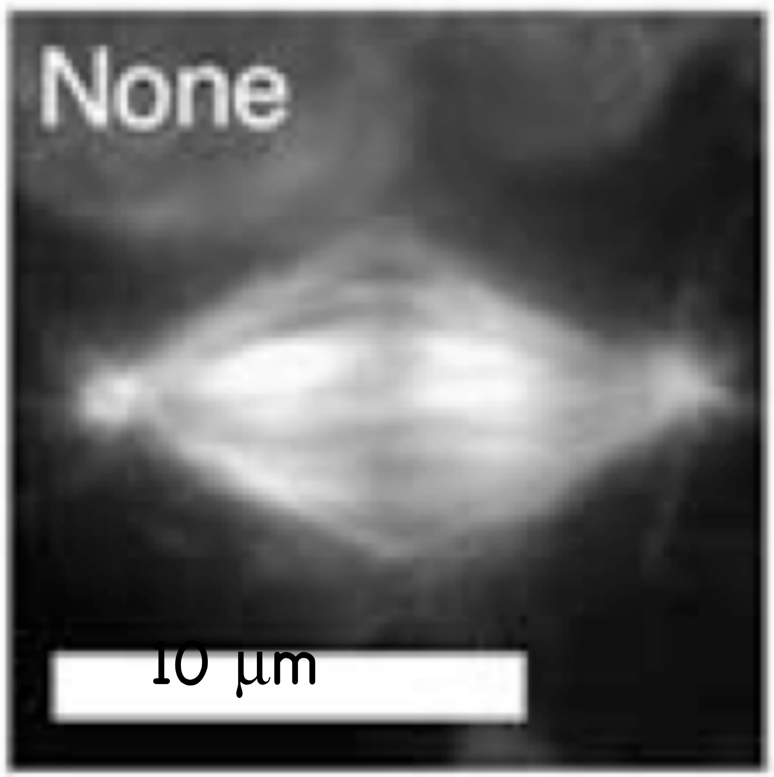
Mitosis and meiosis Organelle transport

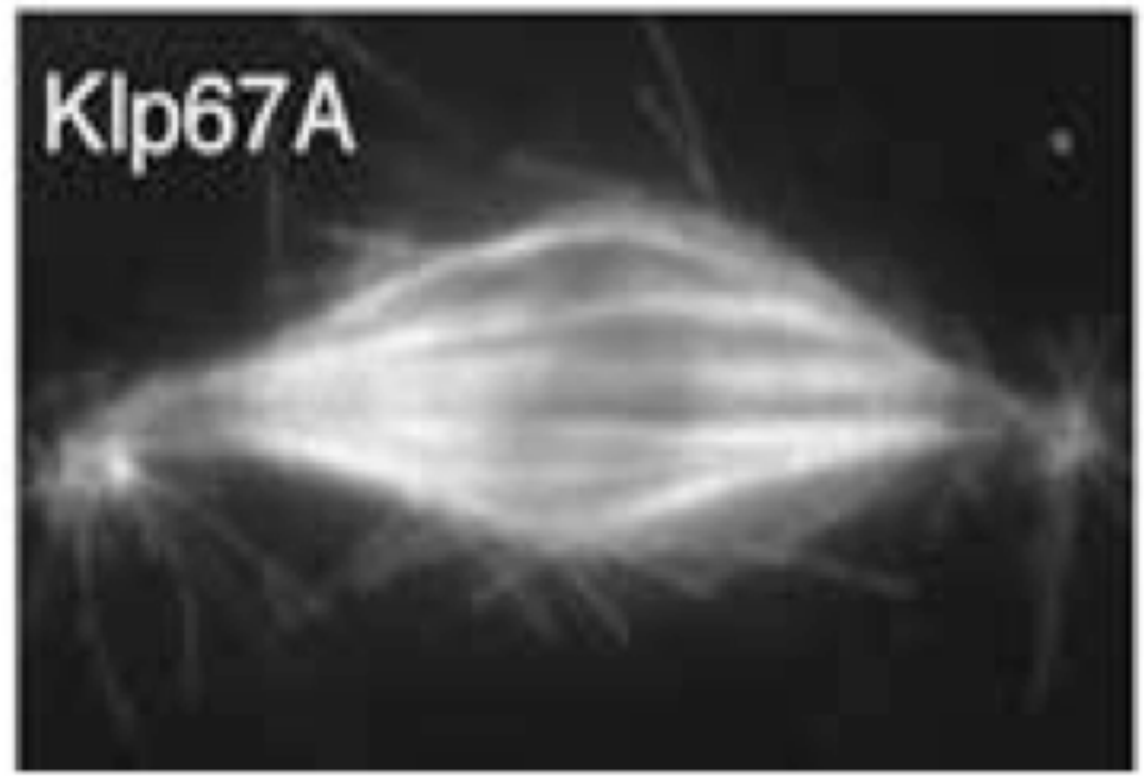
Lawrence et al. 2002

Kinesin-8 regulates the length of the mitotic spindle

Control

Kinesin-8 RNAi



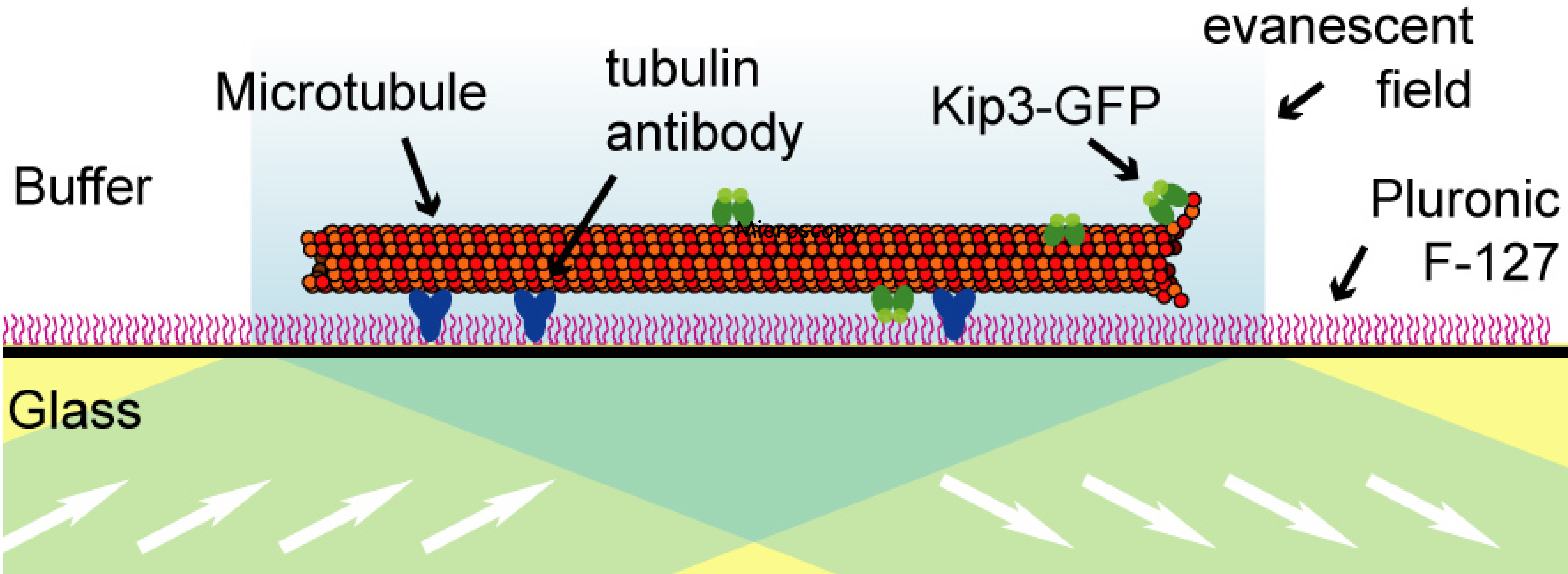


Drosophila S2 cells

Goshima et al. 2005

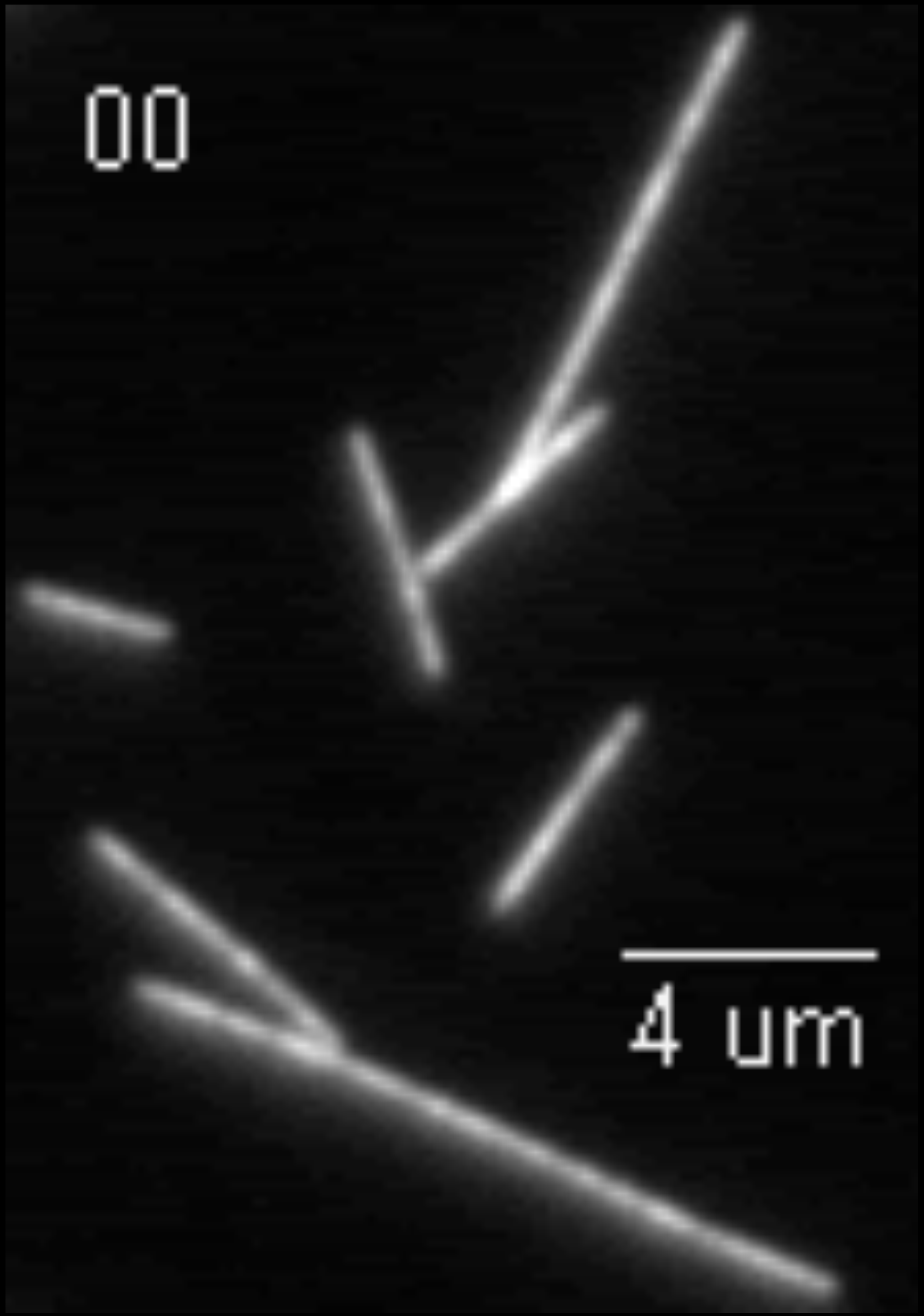
(also true in yeast and human cells)

Depolymerization assay



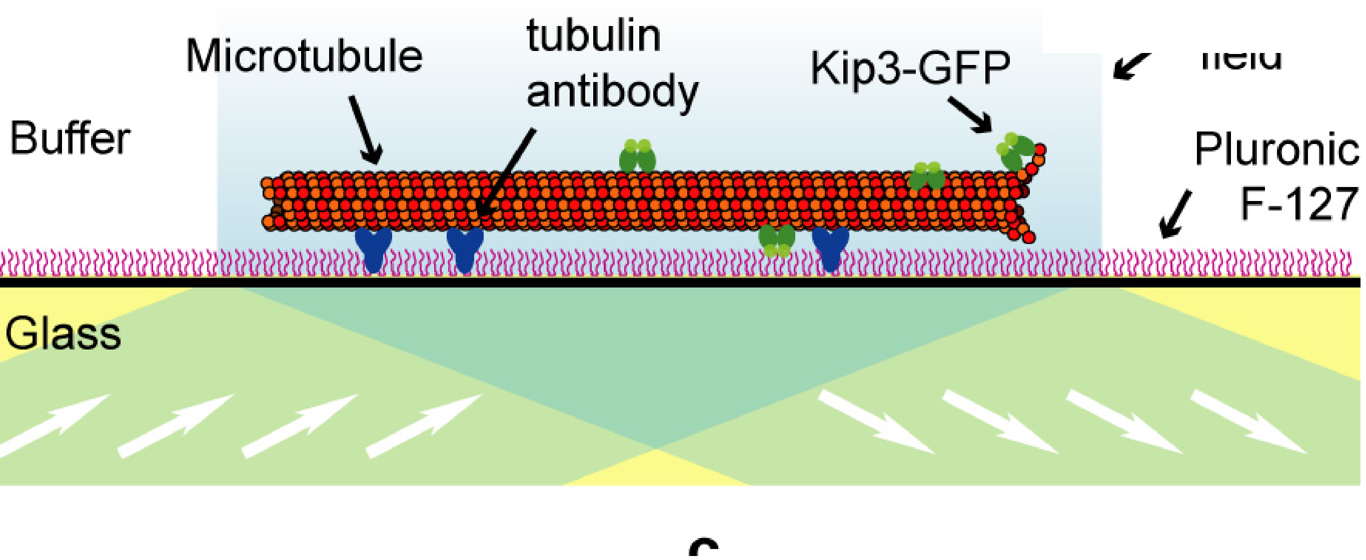
Varga et al. 2006

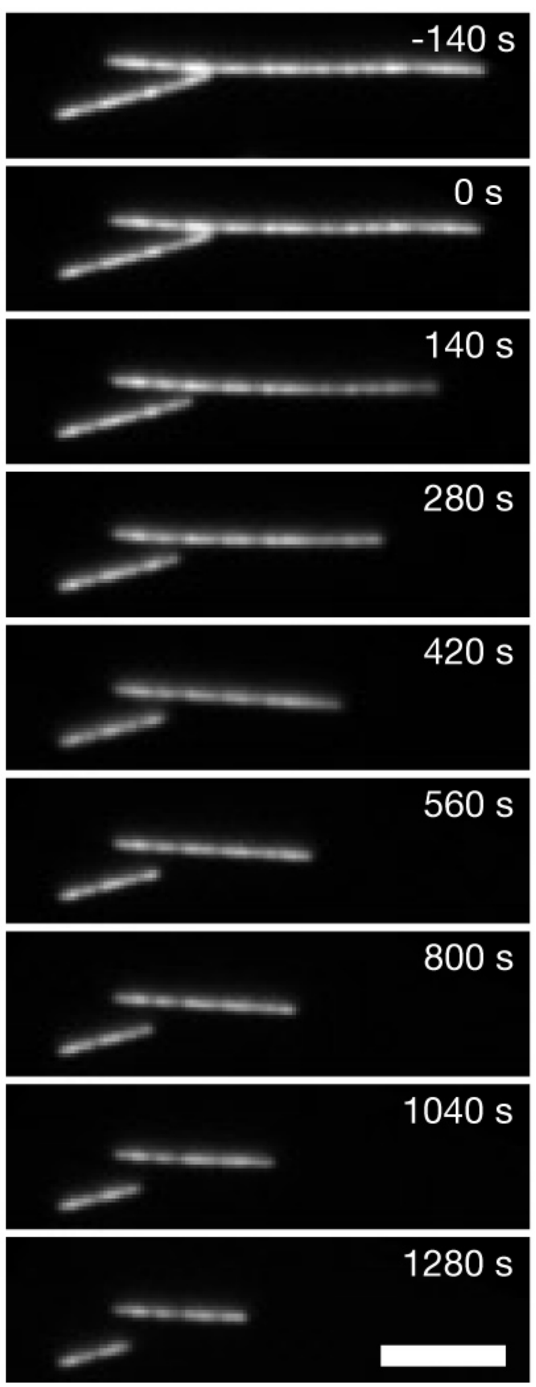
Kinesin-8 is a microtubule depolymerase



GMPCPP-tubulin

Varga et al. 2006



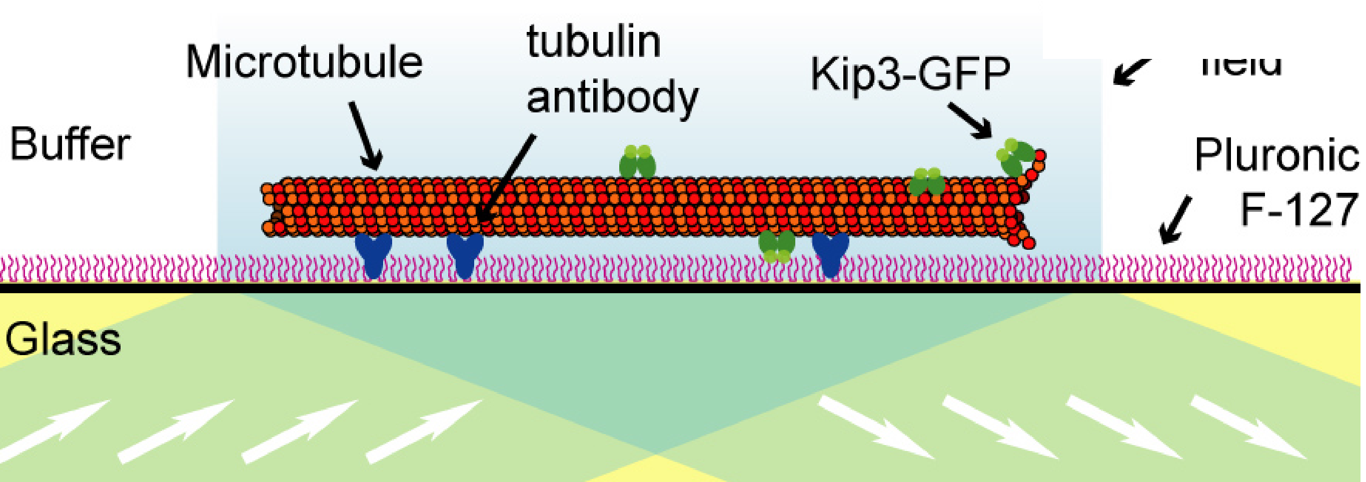


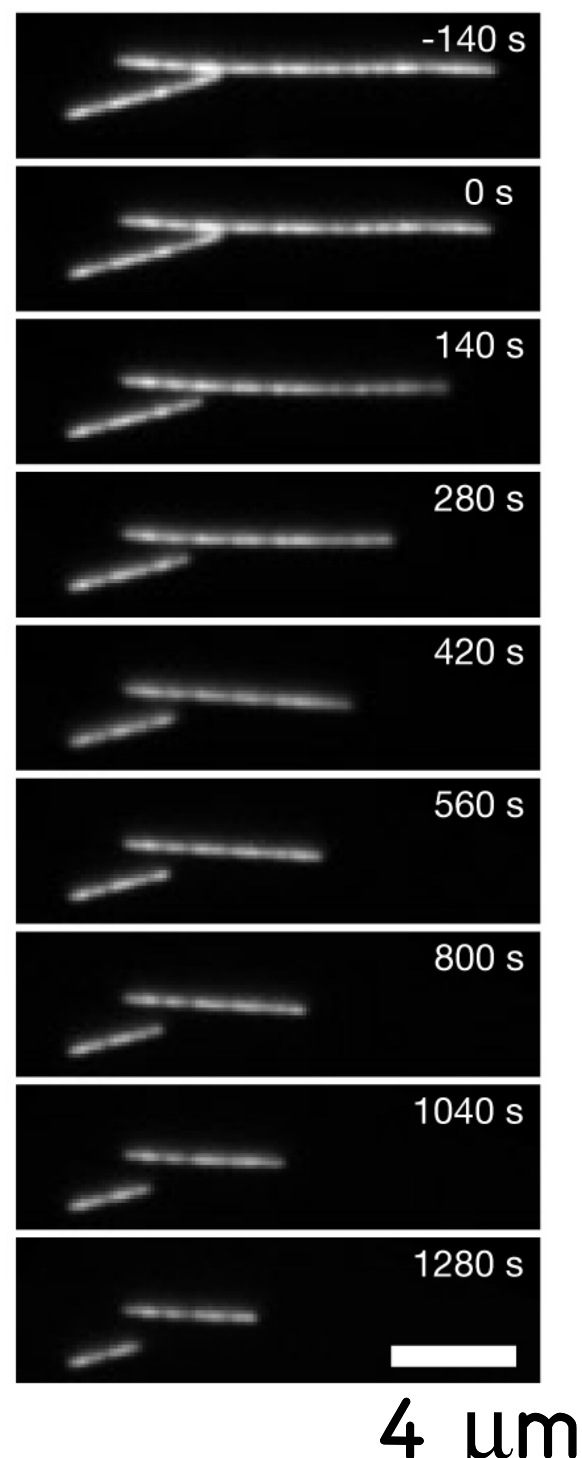
4 μm

Varga et al. 2006

GMPCPP-tubulin

Which end is disassembled?

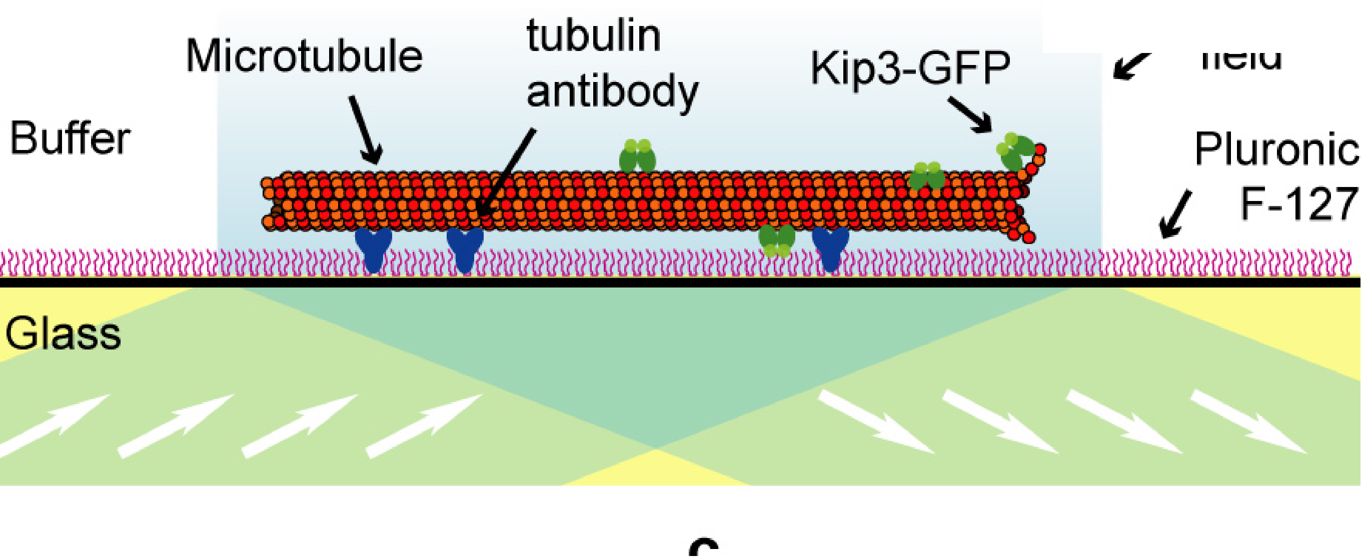


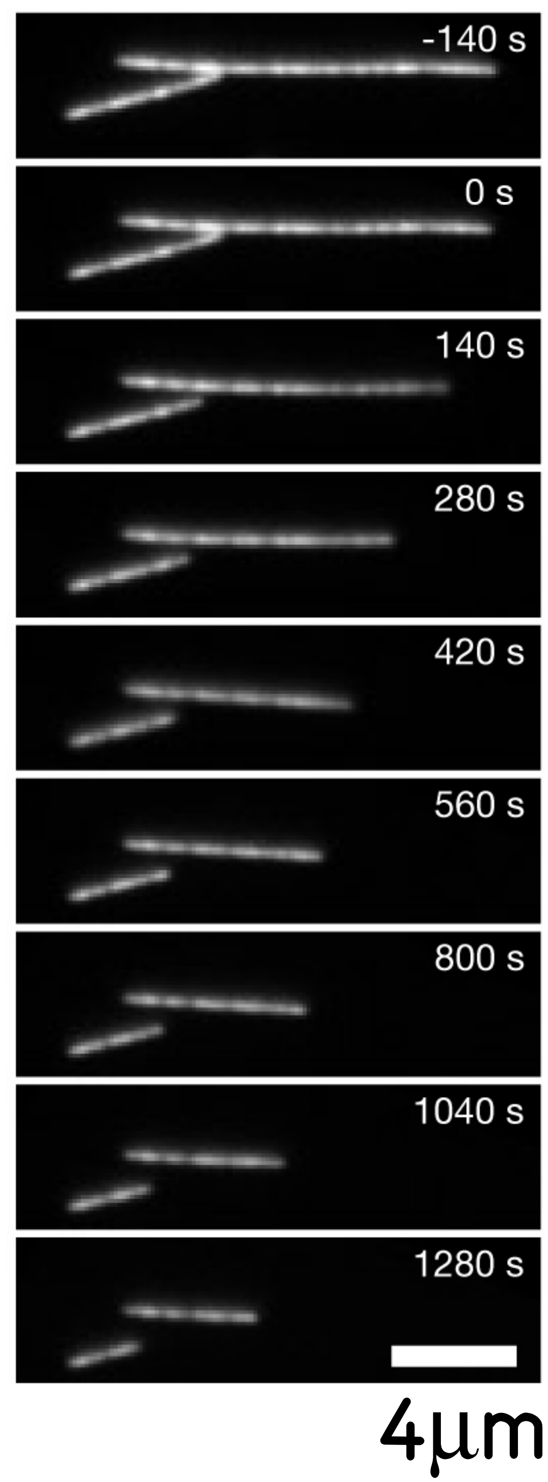


GMPCPP-tubulin

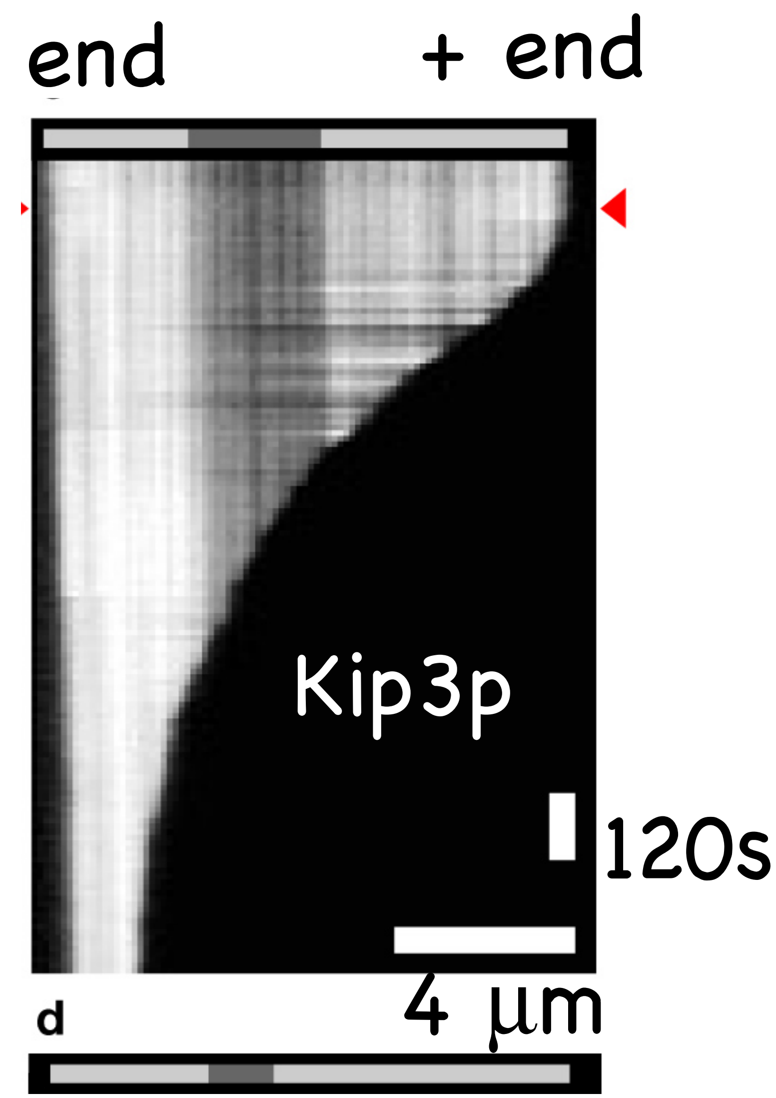
Varga et al. 2006

Which end is disassembled?

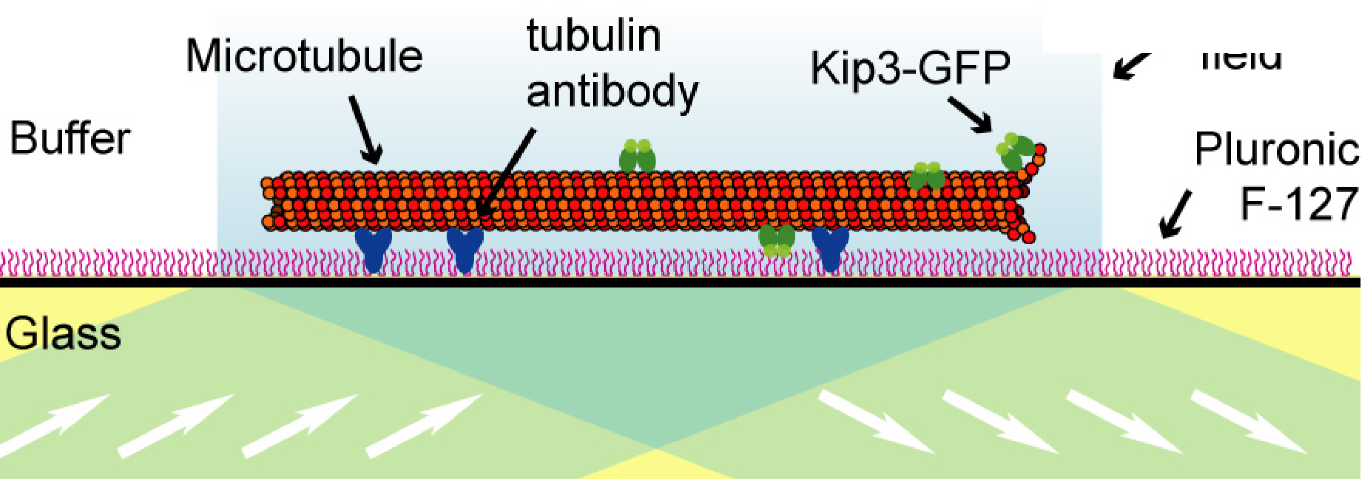


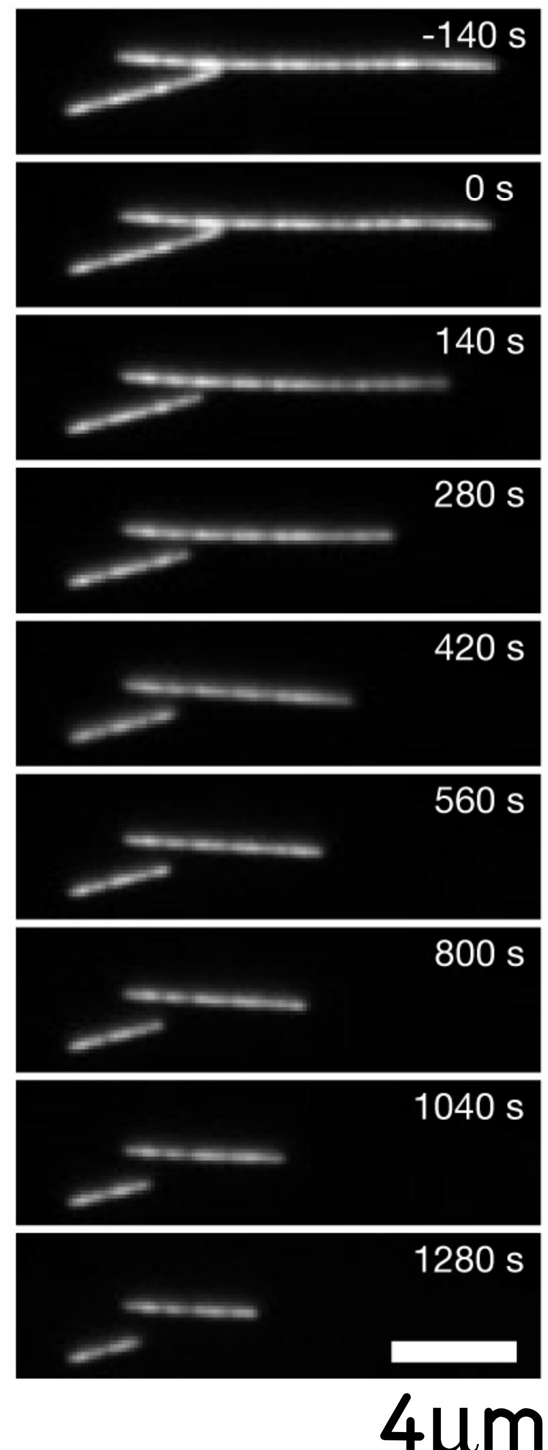


GMPCPP-tubulin

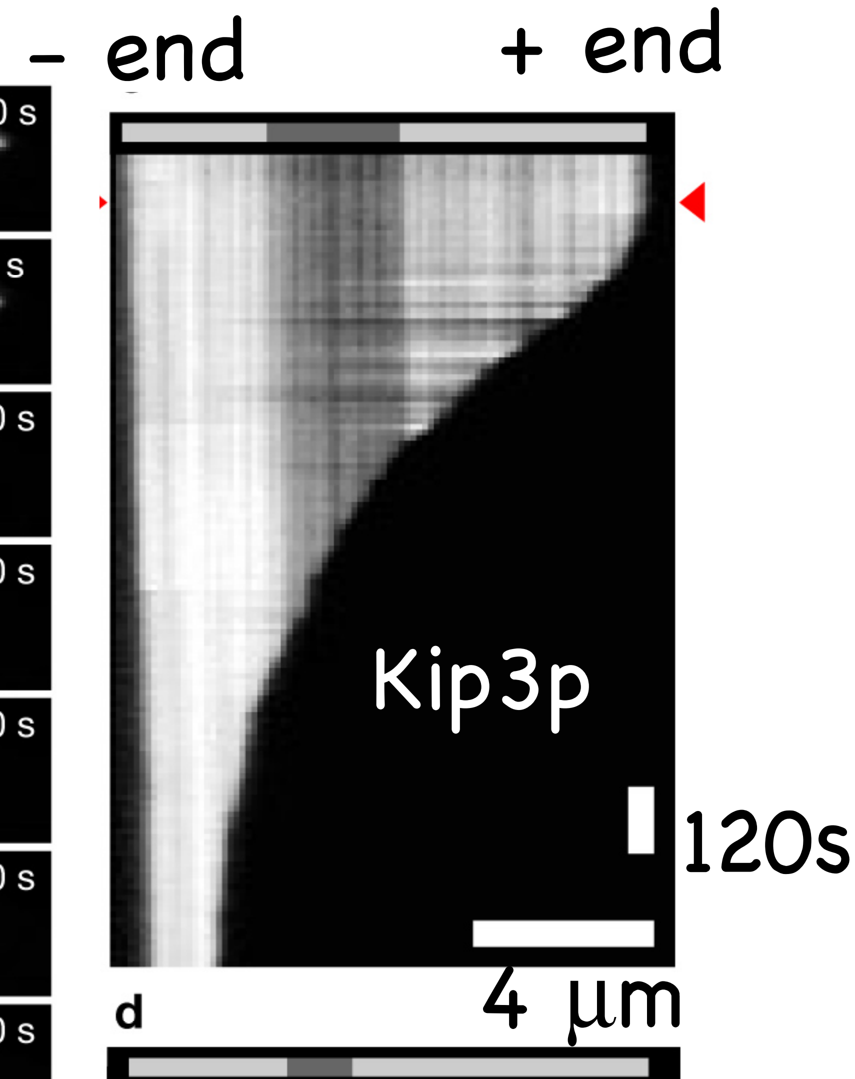


Kinesin-8 is a plus-end depolymerase

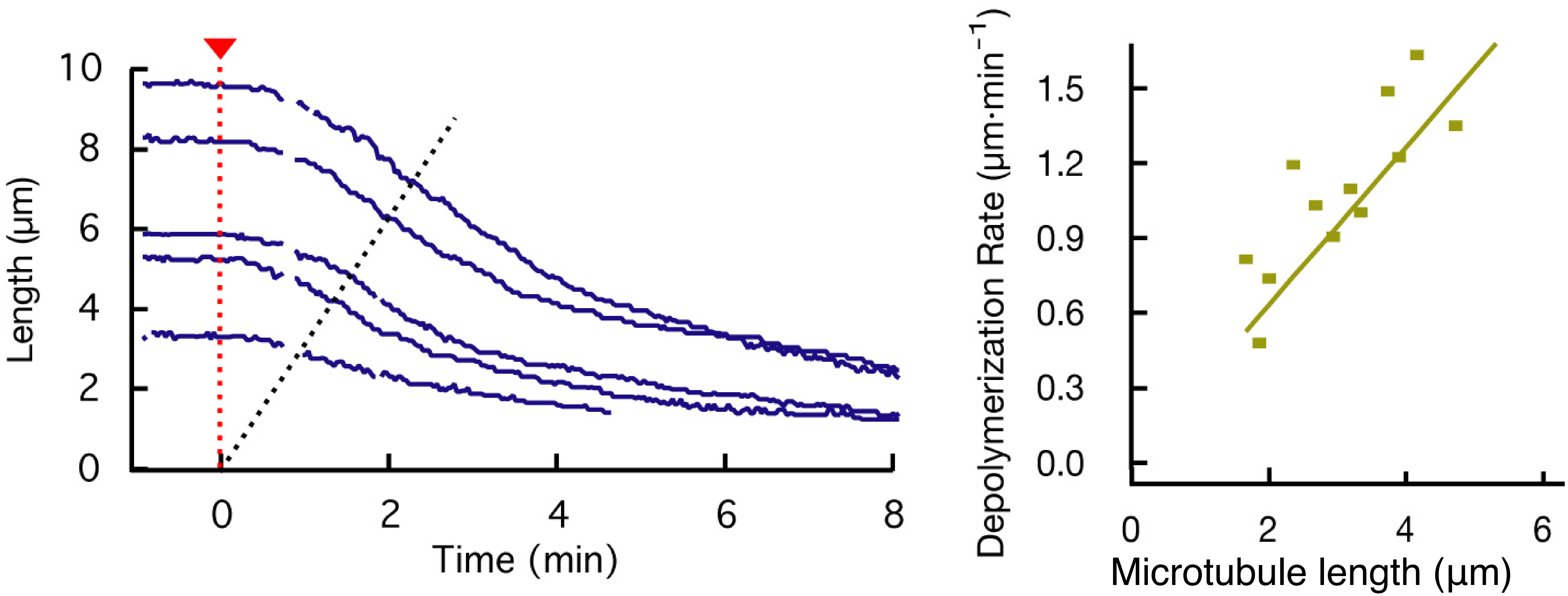




GMPCPP-tubulin



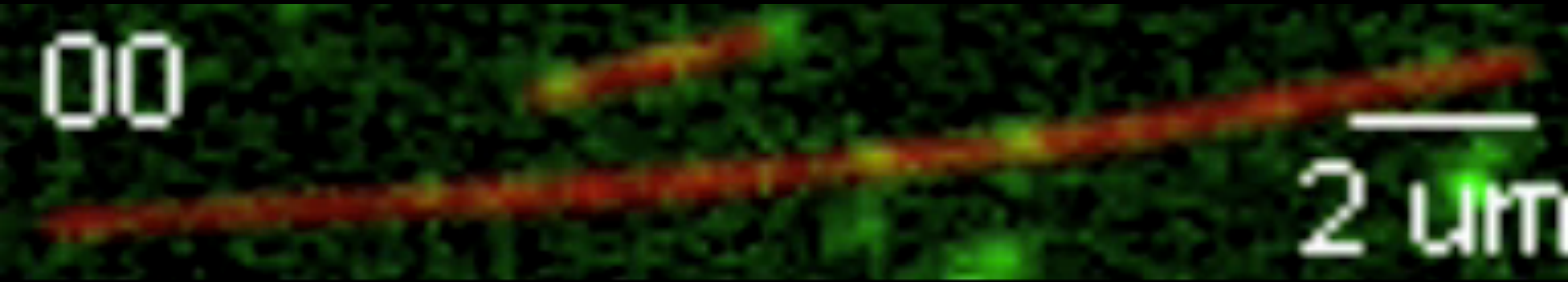
Kinesin-8 depolymerization is length dependent!



How can a small motor (10 nm) know the length of a long microtubule (10 *µ*m)?

Varga et al. 2006

Kinesin-8 is a highly processive motor

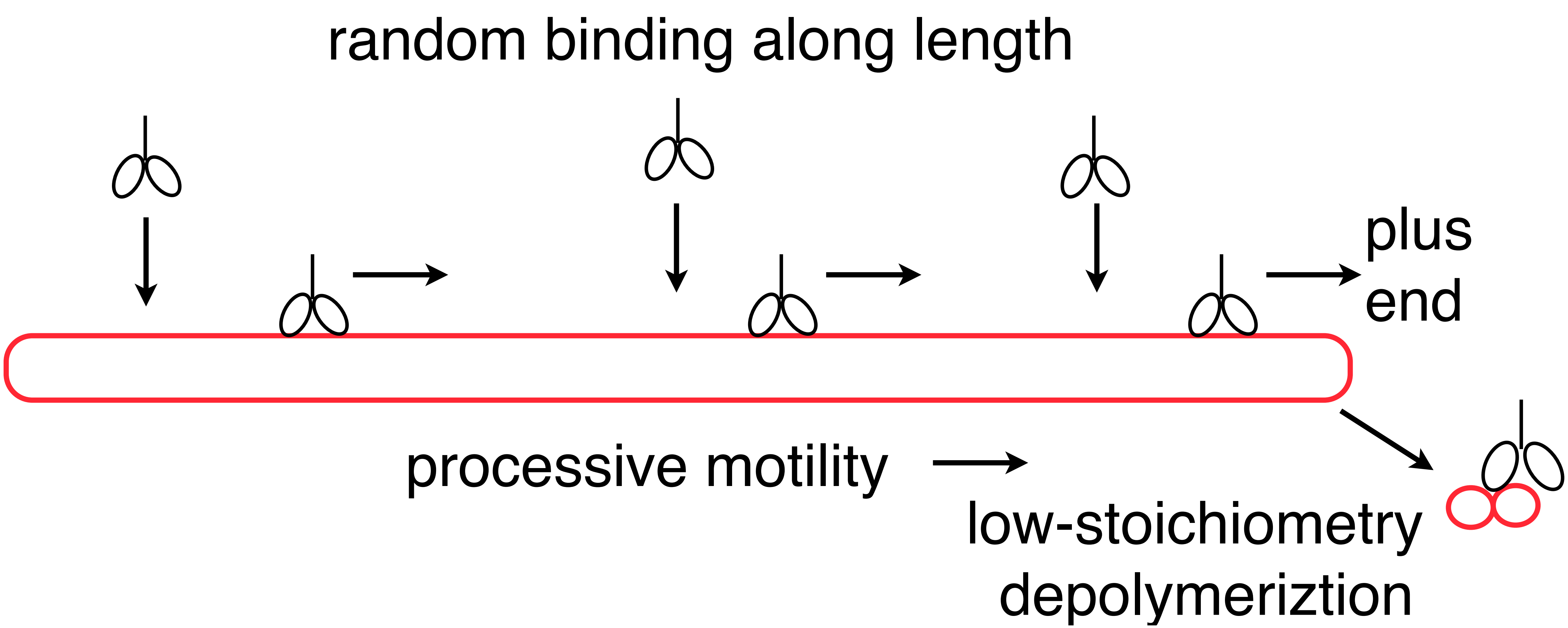


GMPCPP-tubulin

Kip3p-GFP

Varga et al. 2006, 2009

Antenna model



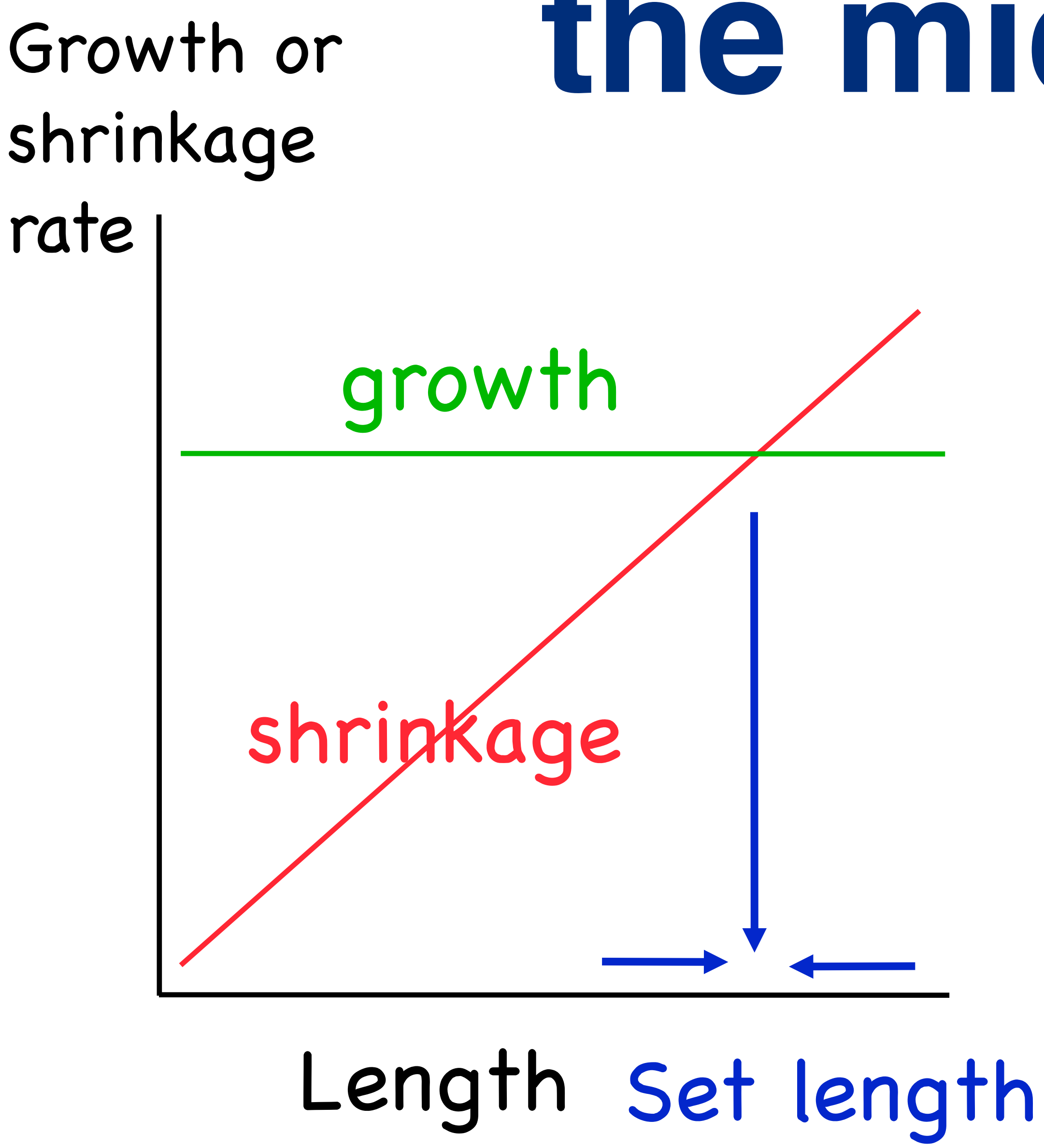
Kinesin-8 is a **processive** motor but a **low-stoichiometry** depolymerase

⇨ length-dependent depolymerization

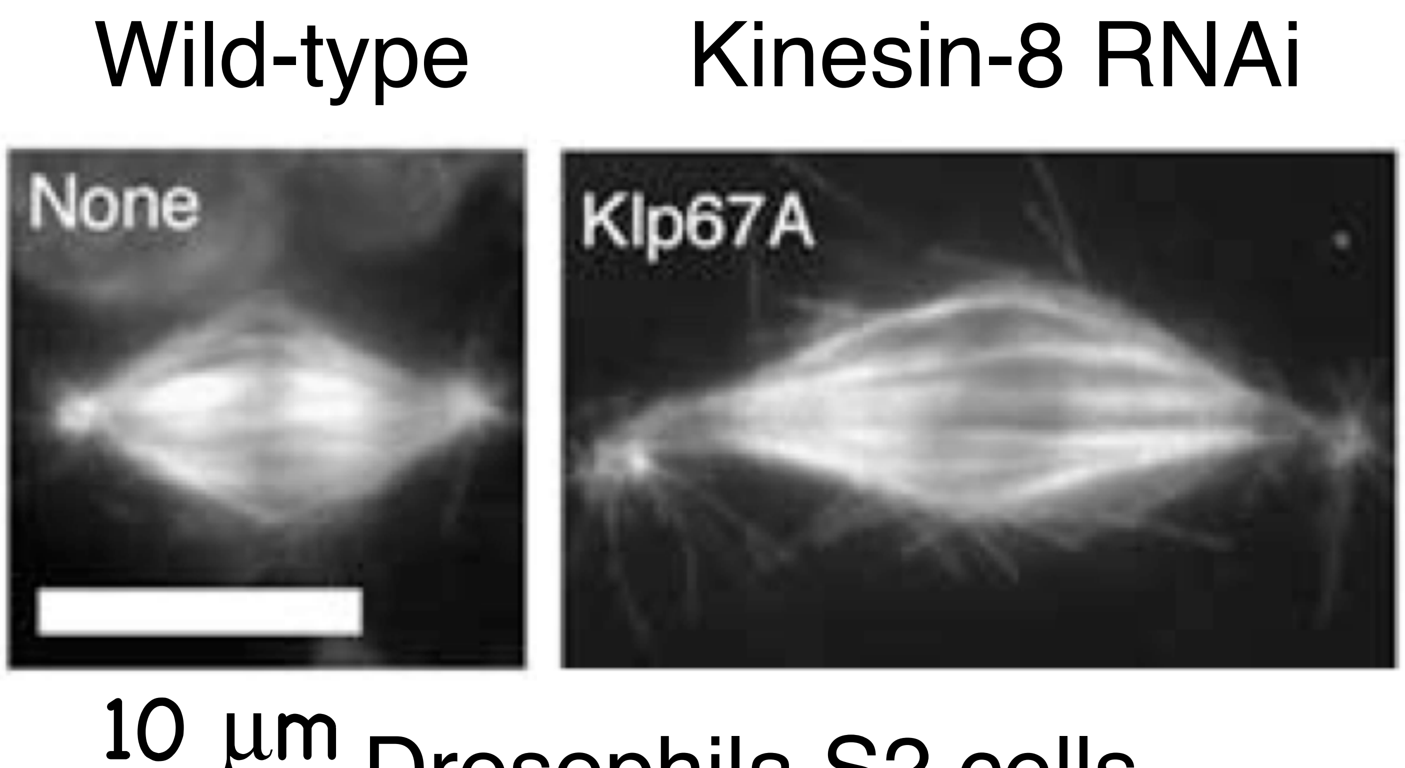
Kinesin-8 measures length by pacing out the microtubule!!

Varga et al. 2009

Hypothesis: Negative feedback by kinesin-8 sets the length of the microtubules!



Howard & Hyman 2007

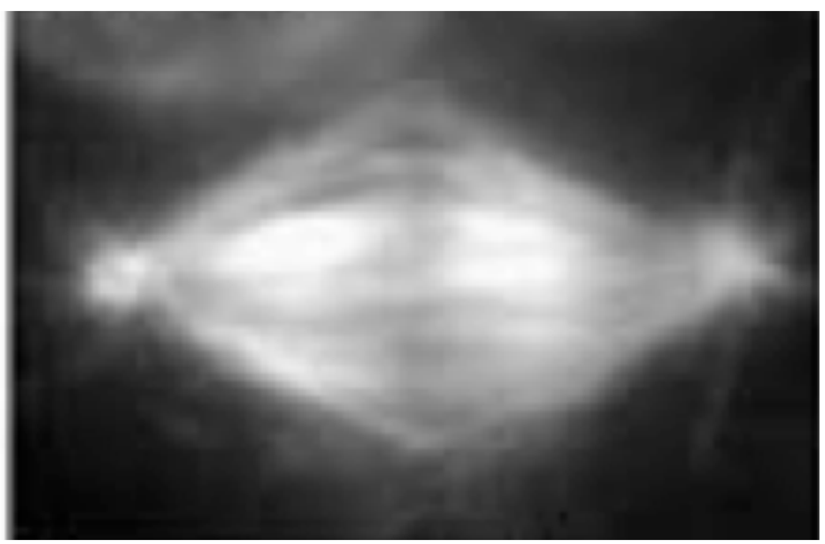


Drosophila S2 cells

Goshima et al. 2005

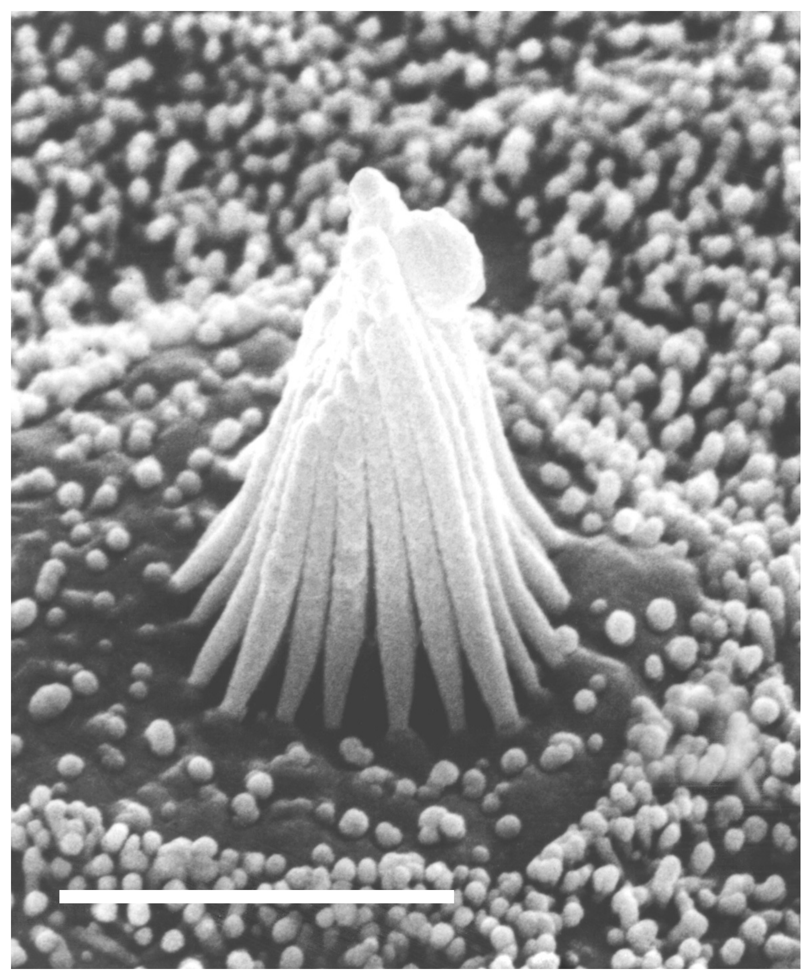
Hypothesis: ensembles of motors perform mechanical computations

Cell division
(spindle length)

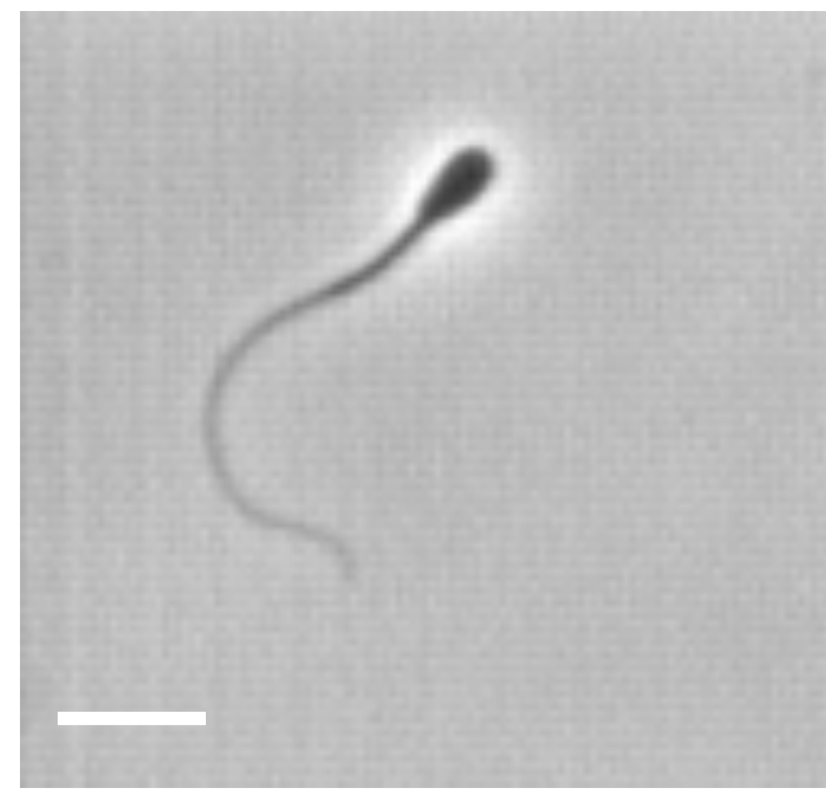


10 µm

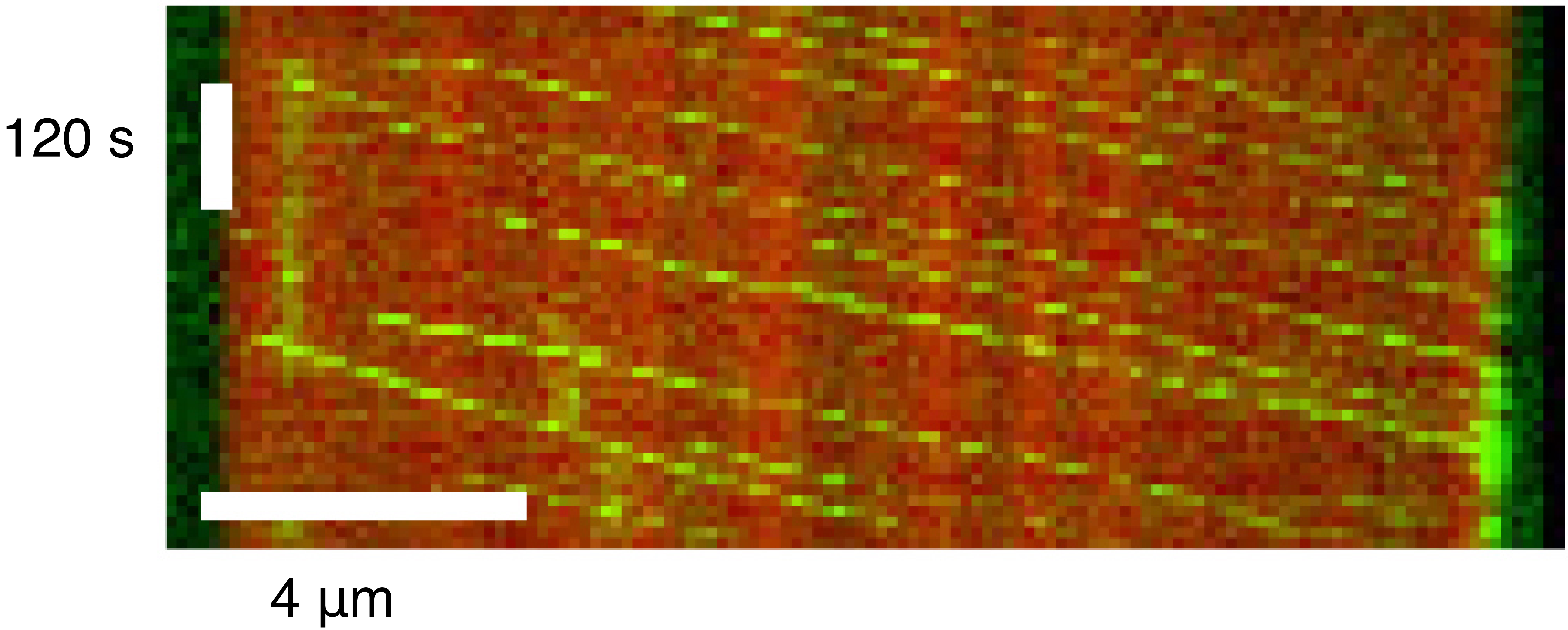
Hearing
(Stereocilia length)



Motility
(Flagella length)



Measuring length is energetically expensive!

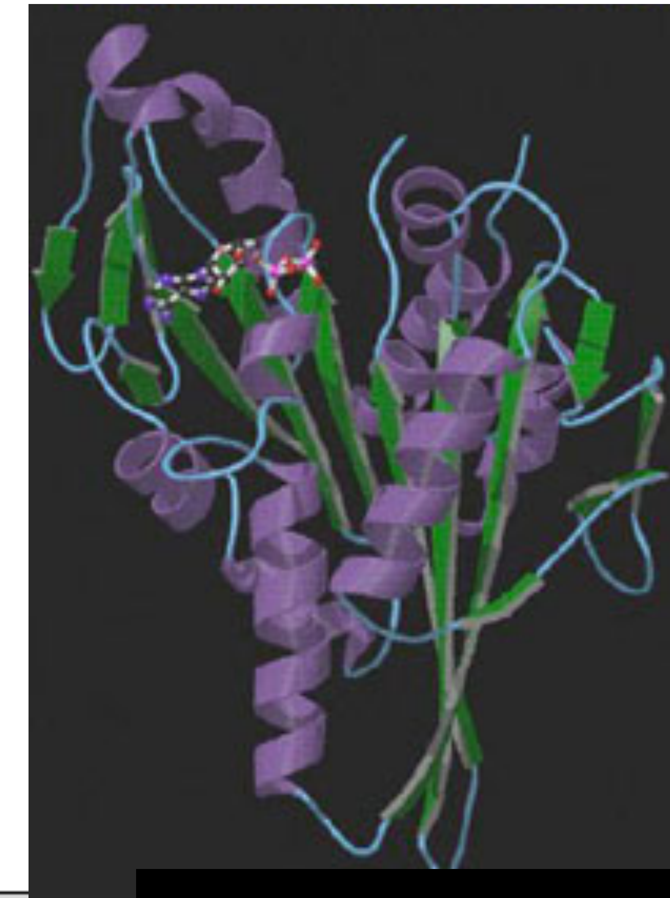


Each 8-nm step costs 1 ATP!
⇒ up to 1000 ATPs per tubulin dimer removed!

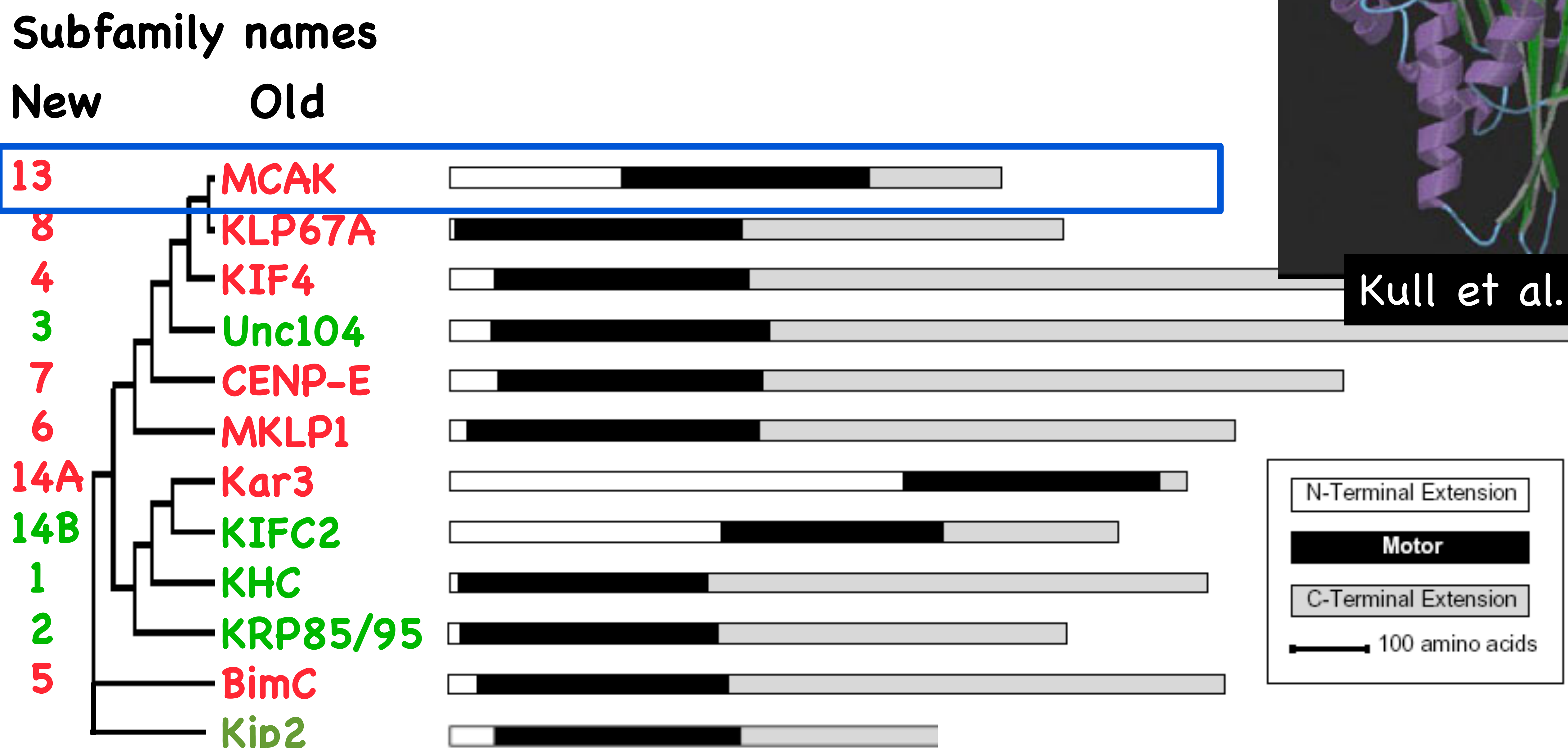
This is VERY expensive!!

Varga et al. 2009

MCAK (Kinesin-13)



Kull et al. 1996



Mitosis and meiosis Organelle transport

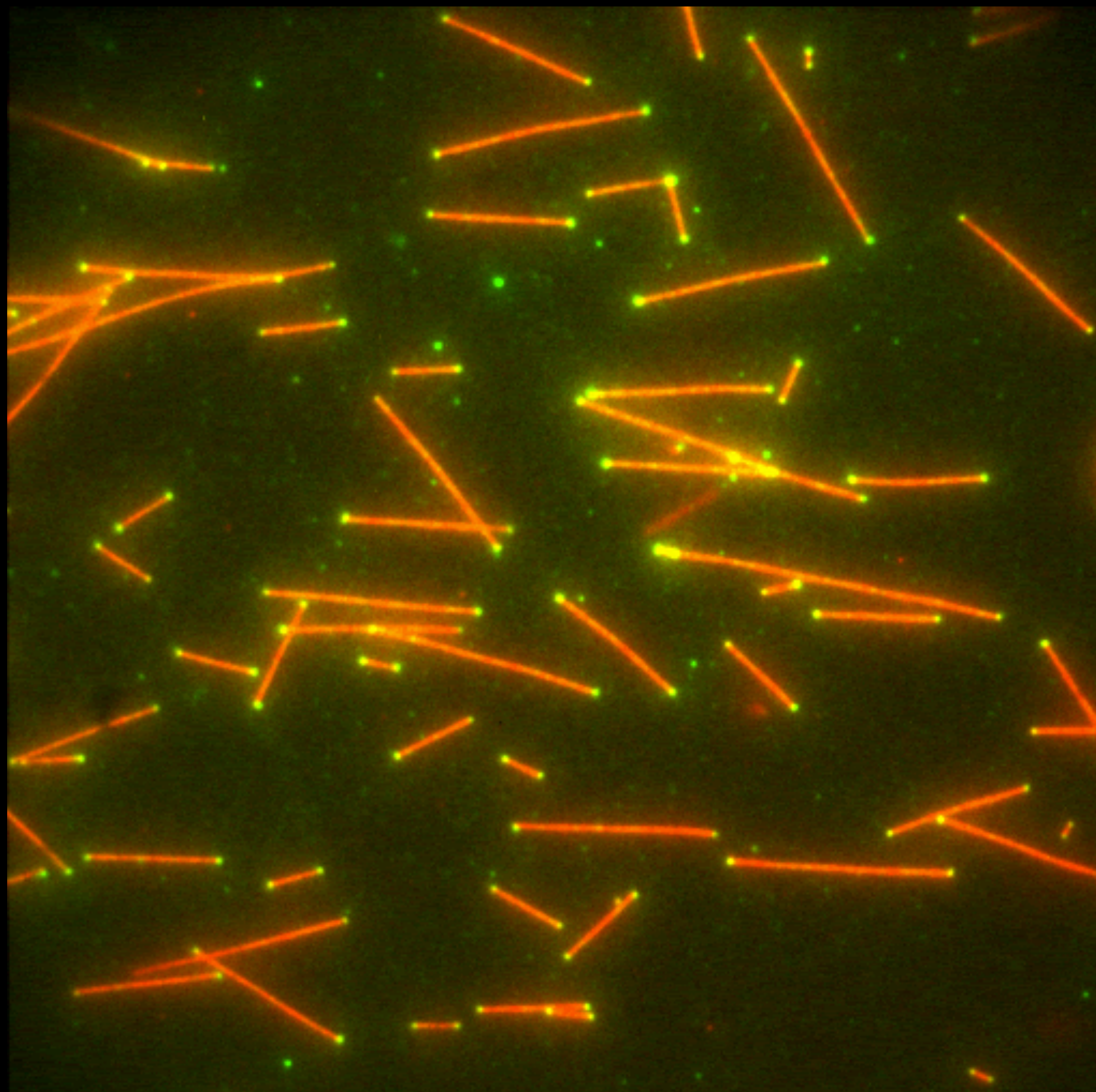
Lawrence et al. 2002

Kinesin-13 depolymerizes microtubules from both ends

Requires ATP hydrolysis!

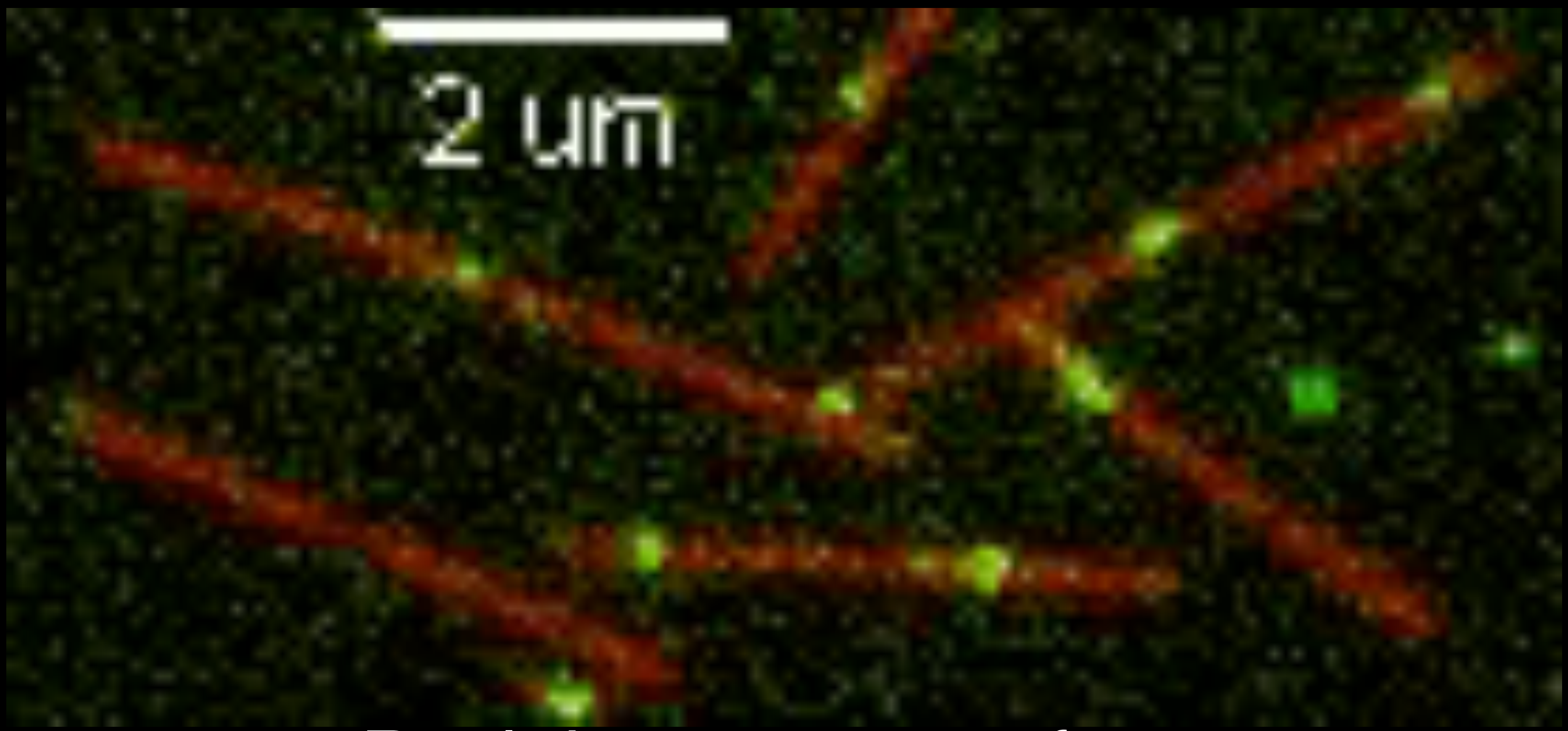
50X time

5 μm



Stefan Diez

Kinesin-13 targets ends by diffusion and capture



Real-time. 0.1 s per frame

No cost to get to the end!!

Only 2-5 ATPs to remove each tubulin dimer

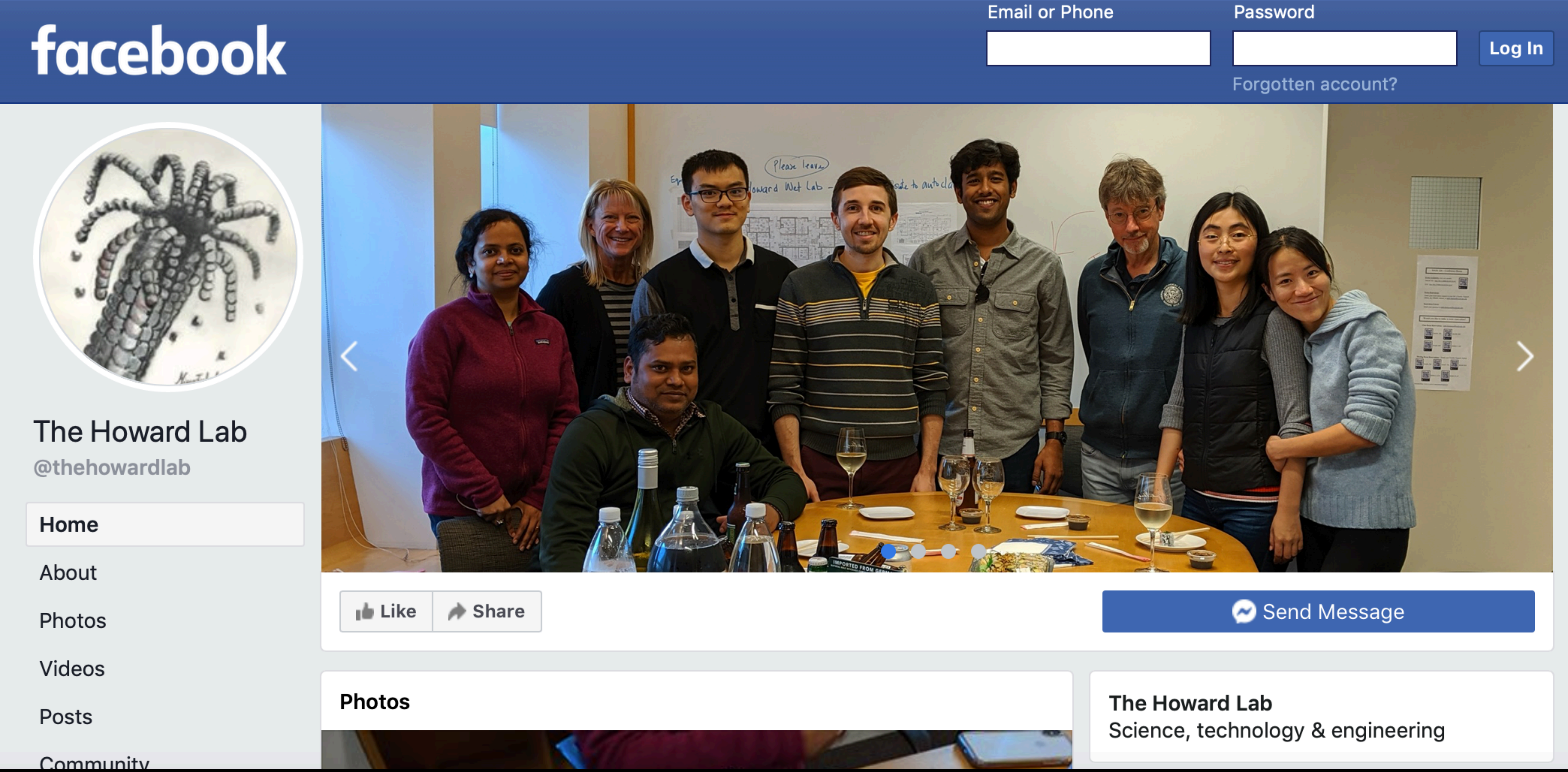
Kinesin-13 is a very “efficient” depolymerase!!

Helenius & Brouhard et al. 2006

General question: how much do cells pay for information?

We have really no idea!

Information and computation are very expensive relative to housekeeping functions



Present members

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Ivy Huang, Kip2
Sonal Shree, dendrites
Sabya Sutradhar, dendrites
Olivier Trottier, dendrites

Former members

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Gary Brouhard, McGill U.
Maria Zanic, Vanderbilt U.
Melissa Gardner, U. Minnesota
Vlado Varga, Czech Academy of Sciences
Erik Schäffer, U. Tübingen
Anneke Hibbel, U. Zurich